THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 134 SEPTEMBER 1, 1941 No. 2

BLOOD VOLUME CHANGES IN MEN EXPOSED TO HOT ENVIRONMENTAL CONDITIONS FOR A FEW HOURS[1]

NATHANIEL GLICKMAN, FORD K. HICK, ROBERT W. KEETON AND M. M. MONTGOMERY

From the University of Illinois College of Medicine, Department of Medicine, Chicago, Ill.

Accepted for publication June 19, 1941

Studies of blood volume changes occurring in various environments made by the use of indirect methods (1, 2, 3) have yielded somewhat conflicting results. With the carbon monoxide and dye methods (4, 5, 6) increases in blood volume have been noted in subjects exposed to warm environments for appreciable lengths of time. Decreases occurred when they were returned to cool surroundings. Other workers have found either small (7) or no changes (8, 9) in blood volumes of subjects after acclimatization to warm environments. The authors (10) have reported increases in plasma volume of subjects exposed to hot environments for 2 to 3 hours. The control experiments, under comfortable conditions, were done 7 to 10 days prior to the day of the experiment. This work was open to the criticism that the physiological states of the individuals might have been altered in the interval. It seemed wise to repeat the experiments measuring the control blood volume on the day of the experiment.

EXPERIMENTAL PROCEDURES. The plan called for two blood volume determinations to be carried out on the same subject the same morning; the first in a comfortable environment; the second after exposure to heat.

Subjects. The subjects were all healthy male medical students ranging from 20 to 27 years of age. They had slept in the room the night before under comfortable conditions. Prior to entrance they drank freely of water. All determinations were carried out on the nude subjects under basal conditions. When the subject was weighed this was done immediately after each blood volume was completed. As a precaution (11, 12)

[1] This work was made possible by a grant of the American Society of Heating and Ventilating Engineers.

the subject was again recumbent for at least 90 minutes before the second blood volume was started. Correction was made for the blood withdrawn.

The room. Conditions in the experimental room were first 28.6°C. dry bulb and 19.7°C. wet bulb. This constituted the "comfortable environment" which is in the "neutral zone" of DuBois (13). Air movement was constant and minimal, and the temperature of the walls was almost identical with the temperature of the air. After the first blood volume determination, the thermostat and humidistat were changed to the desired "hot" readings. Usually about 45 to 55 minutes were needed for the room to warm up. At this time the surface temperature of the globe thermometer was within 0.4°C. of the air temperature.

The temperatures of the hot environments were varied but had in common a dry bulb as high as the normal body temperature, or higher. This compelled the heat loss to be entirely evaporative.

Observations. The blood volumes were determined by the method of Gibson and Evelyn (14) using the dye T1824 and the Cenco-Sheard photelometer. Additional observations, which included blood counts, blood protein analyses, rectal temperature, and vital capacity were all made in the comfortable and again in the hot conditions.

Results. *Controls.* Table 1 shows the results in 5 subjects on whom blood volume determinations were carried out twice in the same morning, the conditions being identical. These experiments show excellent agreement.

Plasma volume increased. Table 2 shows those instances in which an increase in the circulating plasma volume of 5 per cent or more was observed. One notices that the extent of increase in circulating plasma volume may be considerable, reaching 13 per cent which is more than four times the maximum variability observed in the control series. The volume of red cells in circulation also increased appreciably. These increases occurred despite the loss of significant quantities of body water. The wet bulb was rather low in all but one experiment (27.5°C.). In an atmosphere at this wet bulb and a dry bulb of 37.3° to 37.8° gross sweating will appear. Hence the environments used were those conducive to a ready evaporative loss of heat. The heat produced was almost completely lost as indicated by the slight changes in rectal temperature. Apparently mobilization of fluids is an early adjustment for heat elimination. The fluids consist of blood which is presumably stored in the so-called "blood depots," the capillaries of the spleen, liver, lungs, and probably muscles.

Plasma volume decreased. In four experiments a diminution in circulating plasma volume of more than 5 per cent was observed (table 3). It is easy to believe that in the last two experiments on this table, the subjects were in a relative state of anhydremia resulting from exposure of more than three and one-half hours to very hot (44.7°C. dry bulb) conditions with a

TABLE 1

Controls

SUBJECT AND DATE	MINUTES BETWEEN DYE INJECTIONS	PLASMA VOLUME	RBC VOLUME
		cc.	*cc.*
M. A.	0	3497	2896
12/10/39	140	3498	2839
J. W.	0	2976	2747
12/20/39	149	2966	2679
F. R.	0	2083	1699
1/2/40	153	2042	1691
C. N.	0	3226	2453
1/8/40	138	3179	2370
P. H.	0	2941	1403
1/9/40	129	2861	1473

TABLE 2

Plasma volume increased

SUBJECT AND DATE	CONDITIONS		EXPOSURE TIME	PLASMA VOLUME	PER CENT CHANGE	RBC VOL.	PER CENT CHANGE	RECTAL TEMP.	WEIGHT LOSS
	Dry bulb	Wet bulb							
	°C.	°C.	*minutes*	*cc.*		*cc.*		°C.	*grams*
C. G.	Comf.*			3049		2485		36.8	
6/3	37.2	22.1	130	3448	+13.1	2731	+9.9	37.4	
A. M.	Comf.			2583		2349		37.2	
9/23	37.6	27.5	59	2866	+10.9	2742	+16.7	37.2	381
F. C.	Comf.			2840		2232		36.6	
9/5	37.3	24.2	162	3122	+9.9	2589	+16.0	36.9	596
H. C.	Comf.			3367		2468		36.9	
6/7	37.2	24.3	138	3663	+8.8	2855	+15.7	37.4	
F. C.	Comf.			3380		2575		36.3	
7/26	44.7	26.4	216	3572	+5.7	2832	+10.0	36.9	
H. C.	Comf.			3012		2802		37.0	
5/28	37.2	20.1	160	3165	+5.4	2924	+4.4	37.5	

* Comf. = Dry bulb 28.6, wet bulb 19.7.

wet bulb reading of 27.1° and 27.5°C. However, the fall in circulating plasma volume is rather low, less than the increases reported above. The

instance of R. G. on 8/30, when comparatively short exposure of 156 minutes to a moderately hot environment (37.3°C. dry bulb, 20.3°C. wet bulb) led to a fall in blood volume without a rise in rectal temperature of over 37.0°C., is difficult to understand. Further studies will be required to understand the conditions under which plasma concentration occurs following relatively short exposures to hot environments.

Plasma volume unchanged. Table 4 shows results in many cases having in common the observation of less than 5 per cent change in circulating plasma volume. The chief fact obvious at a glance is that the extent of change in plasma volume is not related to the quantity of water lost by sweating. One man lost 1104 grams in weight while showing a change

TABLE 3

Plasma volume decreased

SUBJECT AND DATE	CONDITIONS		EXPOSURE TIME	PLASMA VOLUME	PER CENT CHANGE	RBC VOL.	PER CENT CHANGE	RECTAL TEMP.	WEIGHT LOSS
	Dry bulb	Wet bulb							
	°C.	°C.	*minutes*	*cc.*		*cc.*		°C.	*grams*
S. J.	Comf.			3164		2137		36.4	
9/27	37.5	29.7	69	2977	−5.9	2042	−4.4	37.2	450
R. G.	Comf.			3196		2583		36.6	
8/30	37.2	20.3	156	2995	−6.3	2463	−4.6	36.9	546
R. G.	Comf.			3547		2732		36.7	
7/24	44.7	27.1	216	3336	−6.0	2983	+9.2	37.6	
A. M.	Comf.			3333		2876		36.9	
7/22	44.6	27.5	226	3119	−6.4	2960	+2.9	38.1	

of about the same magnitude in circulating plasma volume as that shown by another subject after losing only 569 grams.

Apparently, the experiments detailed in this table show that the two physiological processes, which produce opposite effects on the blood volume, may be so combined as to neutralize each other. Thus the expansion of the volume by the flow of blood from the reservoirs goes on simultaneously with the evaporation of water from the plasma spread out in the capillaries. By selecting conditions one or the other process may predominate and give an increase or decrease in volume. More commonly no change is noted.

As the exposure to the hot environment continues, new supplies of water are requisitioned from the tissues to replace that evaporated from the plasma. The quantity of water so transferred is indicated as weight loss in grams. Once the blood volume has been increased with the addition

TABLE 4

Plasma volume unchanged

SUBJECT AND DATE	CONDITIONS		EXPOSURE TIME	PLASMA VOLUME	PER CENT CHANGE	RBC. VOL.	PER CENT CHANGE	RECTAL TEMP.	WEIGHT LOSS
	Dry bulb	Wet bulb							
	°C.	°C.	*minutes*	cc.		cc.		°C.	*grams*
S. J.	Comf.			3105		2251		36.7	
9/11	37.8	18.7	169	3140	+1.1	2372	+5.4	37.2	652
R. G.	Comf.			3817		3249		36.8	
8/23	37.4	20.6	160	3725	−2.4	3162	−2.7	37.0	569
J. W.	Comf.			3497		2815		36.9	
5/31	38.0	20.6	91	3546	+1.4	2820	+0.2	37.3	
T. S.	Comf.			3690		3094		36.4	
9/3	37.5	22.4	161	3821	+3.6	3322	+7.4	37.1	554
J. W.	Comf.			3322		2606		36.7	
6/10	37.2	22.7	148	3333	+0.3	2760	+5.9	37.1	
A. M.	Comf.			3185		2859		36.8	
8/14	37.6	25.6	186	3236	+1.6	2951	+3.2	37.4	816
T. S.	Comf.			4255		3044		36.5	
10/1	37.5	27.0	60	4429	+4.1	3217	+5.7	37.1	335
W. H.	Comf.			2398		2484		36.8	
9/19	37.6	28.1	132	2426	+1.2	2632	+6.0	37.3	652
R. G.	Comf.			3164		2601		36.6	
7/16	37.3	28.3	205	3259	+2.9	2822	+8.5	37.2	
A. M.	Comf.			3108		2757		37.0	
8/8	37.6	28.6	201	3114	+0.2	2877	+4.4	37.8	
C. G.	Comf.			3303		2497		36.8	
6/21	37.3	29.7	182	3354	+1.5	2613	+4.6	37.6	
H. C.	Comf.			3509		2930		36.9	
6/24	37.8	30.1	201	3496	−0.4	3075	+5.0	37.9	
T. S.	Comf.			4423		3169		36.7	
10/23	44.2	21.6	187	4277	−3.3	3241	+2.3	37.4	1104
S. J.	Comf.			3154		2383		36.7	
10/25	44.3	23.5	188	3039	−3.6	2320	−2.6	37.6	1279

of new blood from the body reservoirs, the cells remain even though the plasma may be subsequently reduced by evaporation. This is shown by a

significant increase in the circulating volume of red cells in 10 out of 14 experiments in the plasma unchanged group.

Plasma volume increased, unchanged, and decreased in two subjects. In two subjects (table 5) the plasma volume was found to show an increase, no change, and a decrease. The increases occurred when the subjects had been exposed through relatively short periods. The decreases were noted after longer exposures to more severe conditions.

TABLE 5

Plasma volume increased, unchanged and decreased in two subjects

SUBJECT AND DATE	CONDITIONS		EXPOSURE TIME	PLASMA VOLUME CHANGE	PER CENT CHANGE	RBC VOL. CHANGE	PER CENT CHANGE	PER CENT PROTEIN	TOTAL CIRCULATING SERUM PROTEIN	RECTAL TEMP.	WEIGHT LOSS
	Dry bulb	Wet bulb									
	°C.	°C.	*minutes*	*cc.*		*cc.*				°C.	*grams*
A. M.	Comf.			2583		2349		6.68	173	37.2	
9/23	37.6	27.5	59	2866	+10	2742	+16.7	6.72	193	37.2	381
8/8	Comf.			3279		2757		6.95	228	37.0	
	37.6	28.6	201	3216	−1.9	2877	+4.4	6.57	211	37.8	
7/22	Comf.			3333		2876		6.66	222	36.9	
	44.6	27.5	226	3119	−6.4	2960	+2.9	7.23	225	38.1	
T. S.	Comf.			4255		3044		6.78	289	36.5	
10/1	37.5	27.0	60	4429	+4.0	3217	+5.7	6.69	296	37.1	335
9/3	Comf.			3690		3094		7.06	261	36.4	
	37.5	22.4	161	3821	+3.3	3322	+7.4	6.88	263	37.1	554
2/9	Comf.			4000		3220				36.3	
	37.7	30.7	150	3968	−0.3	3173	−1.4			37.6	488
10/23	Comf.			4423		3169				36.7	
	44.2	21.6	187	4277	−3.3	3241	+2.3			37.4	1104

Blood counts. Counts of red and white blood cells were done in duplicate at approximately the same time the dye was injected for the blood volume determinations The blood was obtained by puncturing the ear lobe. The dilutions and counts were made by the same individual throughout (M. M.). Care was taken to obtain thorough mixing of the cells with the diluting fluid and the pipettes were always rotated immediately prior to depositing the suspension in the counting chamber.

It is apparent that there are no significant changes in red cell count except for the two cases having a relative anhydremia. There are significant alterations in white cell count in 10 out of 24 experiments. Since

the changes were in both directions it is difficult at this time to attribute any significance to them.

Serum proteins. Total serum protein was determined by Van Slyke's manometric micro-Kjeldahl (15) method. The albumin was separated from the globulin by Howe's method (16). In every case in which there was an increase in plasma volume (table 6) and in which serum proteins were determined no significant per cent changes were observed, with one exception (H. C. 6/7). The total circulating plasma proteins expressed in grams showed an increase in all cases except one (H. C. 6/7). This result is in accord with those reported by Barbour, Loomis et al. (3) and

TABLE 6

Plasma volume increased

SUBJECT AND DATE	R.B.C.	W.B.C.	Hb.	PER CENT PROTEINS			TOTAL CIRCULATING SERUM PROTEINS	HEMATOCRIT
				Total	Alb.	Glob.		
	millions	*thousands*					*grams*	
C. G.	5.50	5.90	15.3					44.9
6/3	5.62	6.27	15.4					44.3
A. M.	5.58	7.30	16.3	6.68	4.56	2.12	173	47.6
9/23	5.43	7.35	16.9	6.72	4.61	2.11	193	48.9
F. C.	4.84	7.75	15.8	6.22	4.65	1.57	177	44.0
9/5	5.02	9.80	15.7	6.38	4.60	1.78	199	46.3
H. C.	6.17	8.05	14.3	6.19	4.66	1.53	208	42.3
6/7	6.08	7.95	14.8	5.56	4.30	1.26	204	43.8
F. C.	5.21	6.85	15.0	6.14	4.26	1.88	218	43.2
7/26	5.33	8.20	15.2	6.24	4.49	1.75	223	44.2
H. C.	6.42	4.80	16.7	6.11	4.70	1.41	184	48.2
5/28	6.87	6.75	16.2	6.38	4.40	1.98	202	48.0

Bazett et al. (6). Evidently, the increased plasma volume is composed of fluid having about the same protein concentration as the normal plasma.

The same situation exists in the unchanged group (table 8) where just one subject (A. M. 8/8) shows a drop of questionable significance comprising 5.5 per cent of the initial protein concentration. The grams of total circulating plasma proteins also were unchanged. In the concentration group (table 7) there was a percentage increase in the plasma proteins in two cases without change in the total circulating proteins (expressed in grams). This again indicates that the concentration is attributable to the evaporation of water and should be considered as an anhydremic phe-

nomenon. The results on R. G. 8/30 are not in agreement and at present are not explained.

Changes in pulse rate. The correlation between the pulse rate and the rectal temperatures of subjects in a steady state exposed to hot environments of varying degrees has been previously reported (17). From 101 observations on 5 subjects it was noted that an average increase in pulse rate per degree centigrade rise in rectal temperature was 25 beats. At that time changes in plasma volume were not considered. In table 9 the correlation is now extended to include both plasma volume changes and changes in rectal temperatures. It is evident that an increase in plasma volume tends to minimize the increase in pulse rate. The primary correlation therefore, of pulse rate increase, is with a rise in rectal temperature and the secondary one with plasma volume changes.

TABLE 7

Plasma volume decreased

SUBJECT AND DATE	R.B.C.	W.B.C.	Hb.	PER CENT PROTEINS			TOTAL CIRCULATING SERUM PROTEINS	HEMATOCRIT
				Total	Alb.	Glob.		
	millions	*thousands*					*grams*	
S. J.	4.72	8.57	13.5	6.12	4.60	1.52	194	40.3
9/27	4.39	9.50	13.7	6.28	4.55	1.73	187	40.7
R. G.	4.70	7.90	15.2	6.53	4.86	1.67	209	44.7
8/30	5.00	9.55	15.3	6.00	4.97	1.03	180	45.1
R. G.	5.45	7.35	15.3	6.54	4.80	1.74	232	43.5
7/24	6.23	7.00	16.2	7.29	5.18	2.11	243	47.2
A. M.	5.35	7.38	16.2	6.66	4.81	1.85	222	46.3
7/22	6.11	6.10	17.3	7.23	5.22	2.01	225	48.8

Discussion. The difficulties in the measurements of plasma volume are many. Although these have been reduced by the studies of Rowntree (18), Gregersen (19), Gibson (8) and others (20, 21), one still encounters pitfalls. It can be readily seen that deductions as to plasma volume changes derived by following hemoglobin, specific gravity, total solids, and hematocrit determinations are unreliable. Thus in the experiments in which the plasma showed either a dilution or concentration, there were no significant changes in these values.

With the dye method as now standardized, one would expect to secure over a period of time reproducible plasma volumes in any given subject. Reference to table 5 shows that the plasma volumes of two subjects who should have been in a steady state under our "comfortable environment" were not the same from day to day. Similar discrepancies have been

TABLE 8

Plasma volume unchanged

SUBJECT AND DATE	R.B.C.'S	W.B.C.'S	Hb.	PER CENT PROTEIN			TOTAL CIRCULATING SERUM PROTEINS	HEMATOCRIT
				Total	Alb.	Glob.		
	millions	*thousands*					*grams*	
S. J.	4.72	10.05	14.7	6.42	4.63	1.79	199	42.0
9/11	4.78	7.85		6.68	4.47	2.21	208	43.0
R. G.	5.27	7.70	15.9					46.0
8/23	5.30	7.60	15.9					45.9
J. W.	5.01	7.80						44.6
5/31	5.15	4.50						44.3
T. S.	4.85	5.47		7.06			260	45.7
9/3	4.77	5.40		6.88			263	46.5
J. W.	5.08	7.25	14.8					44.0
6/10	5.22	5.60	15.4					45.3
A. M.	5.38	6.92	16.6	6.76	4.90	1.86	215	47.3
8/14	5.35	6.15	16.4	7.08	5.04	2.04	229	47.7
T. S.	4.69	5.25	14.8	6.78	4.65	2.13	288	41.7
10/1	4.62	4.72	14.9	6.69	4.56	2.13	296	42.1
W. H.	5.27	9.22	17.7					50.9
9/19	5.68	9.10	17.8					52.0
R. G.	5.17	8.00	15.8	6.71	4.72	1.99	212	45.1
7/16	5.27	8.25	16.2	6.72	4.84	1.88	219	46.5
A. M.	5.60	9.47	17.0	6.95	4.73	2.22	216	47.0
8/8	5.35	9.45	16.9	6.57	4.78	1.79	204	48.0
C. G.	5.03	7.12	14.8	6.65	4.57	2.08	220	43.1
6/21	5.32	5.72	15.3	6.84	4.75	2.09	229	43.8
H. C.	5.67	7.45	15.3	6.60	4.66	1.94	231	45.6
6/24	5.74	7.22	15.8	6.79	5.10	1.69	237	46.8
T. S.	4.73	5.20	14.8					41.7
10/23	4.66	6.22	15.3					43.1
S. J.	4.74	9.37						43.0
10/25	4.59	9.70						43.3

found in the literature. This is not necessarily a reflection on the method or the technique of its application. Apparently the circulating plasma

volume in any individual is not necessarily fixed. It is not always the same day after day. There is a constant phasic adjustment between the circulating blood volume and the vascular bed. Patients with large spleens (22) have large blood volumes. After the spleen is removed the volume is decreased. The plasma volume drops (10, 11) when the individual assumes the standing position. When the atmospheric environment is cool the quantity of blood in the lungs as measured by the vital capacity is larger than when the environment is hot (23). When the surroundings are warm the blood shifts to the periphery of the body.

It now becomes apparent that one must exercise extreme caution in interpreting blood volume estimations from day to day, such as have been reported in acclimatization studies. Consequently, we believe our procedure of determining the blood volume immediately before and after an experiment is a more reliable index of a given physiological adjustment. It would seem that in the adjustment to hot environments the plasma volume regularly increases. In a number of our experiments this was

TABLE 9

Pulse rate changes

PLASMA VOLUME	EXPERIMENTS	AVERAGE INCREASE OF PULSE/°C. RISE IN RECTAL TEMPERATURE
Increased	6	7
Unchanged	14	18
Decreased	4	34
Not determined	101	25

found to be between 5 per cent and 13 per cent. In others it was not observed but an increase in red cell volume was noted. In these latter experiments it would seem that water was lost from the plasma, but the cells which had been swept into the circulation during the dilution phase remained behind and were observed as an increased red cell volume. It was further noted that in those subjects whose plasma volume increased there was no significant rise in the pulse as the rectal temperature rose. This suggests that some subjects have a readily mobilizable reserve plasma volume. With the rise in temperature the peripheral vessels dilate and the increased vascular bed is filled with this reserve plasma. Other subjects who are unable to supply adequate plasma, or whose plasma volume is reduced by evaporation, develop a disparity between the blood volume and the vascular bed. An example of this acute disparity was previously reported by the authors (17). The inability of certain individuals to withstand heat may be attributed to the disparity which arises when the plasma volume fails to increase on exposure to hot conditions.

CONCLUSIONS

1. Subjects were exposed to environments having a dry bulb of 37.2 and 44.7 degrees C. and wet bulb temperatures of 20.1 to 27.5 degrees. The globe thermometer showed less than 0.4°C. difference between its surface and the surrounding air. Air currents were minimal. The periods of exposure ranged from 59 to 160 minutes. In 6 experiments there was an increase in circulating plasma volume, red cell mass, and grams of total circulating serum proteins, all of which would be expected on the assumption that the new fluids were contributed by blood from the body reservoirs (spleen, and inactive capillary beds in muscles, lungs, and viscera).

2. In 4 other experiments in which the dry and wet bulbs showed approximately the same ranges, there was a decrease in the circulating blood plasma and variable changes in the red cell mass. The periods of exposure ranged from 69 to 226 minutes. The changes in the plasma proteins, red and white cell counts were such as would be explained on the assumption that water was lost by evaporation from the blood plasma.

3. In 14 experiments on subjects exposed to the same type of environments there were no significant changes in circulating plasma volume, serum proteins, or blood counts. There were slight but definite increases in the red cell volume in 11 out of 14 experiments. The lack of change in plasma volume was attributed to a summation of the two adjustment factors described above which tend to neutralize each other.

4. In steady states the increase in pulse rate correlates well with rises in rectal temperature. If the plasma volume increases, the rise in pulse rate per degree rise in rectal temperature is less than in those subjects whose plasma volume remains unchanged or decreases.

5. A considerable quantity of fluid can be requisitioned from the tissues and evaporated from the blood plasma without affecting the circulating blood volume.

REFERENCES

(1) Hunt, E. H. J. Hyg. **12:** 479, 1912.
(2) Young, W. J., A. Breinl, J. J. Harris and W. A. Osborne. Proc. Roy. Soc. B. **91:** 111, 1920.
(3) Barbour, H. G., N. M. Loomis, R. W. Frankman and J. H. Warner. J. Physiol. **59:** 300, 1924–25.
(4) Barcroft, J., C. A. Binger, A. V. Bock, J. H. Doggart, H. S. Forbes, G. Harrop, J. C. Meakins and A. C. Redfield. Phil. Trans. Roy. Soc. B. **211:** 351, 1923.
(5) Loc. cit. See note 4, page 455.
(6) Bazett, H. C., F. W. Sunderman, J. Doupe and J. C. Scott. This Journal **129:** 69, 1940.
(7) Forbes, W. H., D. B. Dill and F. G. Hall. This Journal **130:** 739, 1940.
(8) Gibson, J. G. II and W. A. Evans, Jr. J. Clin. Investigation **16:** 301, 1937.

(9) TALBOTT, J. H., H. T. EDWARDS, D. B. DILL AND L. DRASTICH. Am. J. Trop. Med. **13:** 381, 1933.
(10) HICK, F. K., R. W. KEETON, N. GLICKMAN AND H. C. WALL. J. A. S. H. V. E. **11:** 50, 1939.
(11) WATERFIELD, R. L. J. Physiol. **72:** 110, 1931.
(12) THOMPSON, W. O., P. K. THOMPSON AND M. E. DAILEY. J. Clin. Investigation **5:** 573, 1928.
(13) DUBOIS, E. F. The mechanism of heat loss and temperature regulation. Lane Medical Lecture, Stanford University Press, 1937.
(14) GIBSON, J. G. II AND K. A. EVELYN. J. Clin. Investigation **17:** 153, 1938.
(15) VAN SLYKE, D. D. J. Biol. Chem. **71:** 235, 1927.
(16) HOWE, P. E. J. Biol. Chem. **49:** 109, 1921.
(17) KEETON, R. W., F. K. HICK, N. GLICKMAN AND M. M. MONTGOMERY. J. A. S. H. V. E. January, 1940.
(18) KEITH, N. M., L. G. ROWNTREE AND J. T. GERAGHTY. Arch. Int. Med. **16:** 547, 1915.
(19) GREGERSEN, M. I. J. Lab. and Clin. Med. **23:** 423, 1938.
(20) MOORE, J. W. AND J. M. KINSMAN. T. A. Am. Physicians **51:** 260, 1936.
(21) SUNDERMAN, F. W. AND J. H. AUSTIN. This Journal **117:** 474, 1936.
(22) ROWNTREE, BROWN AND ROTH. The volume of the blood and plasma. W. B. Saunders Co., Philadelphia, 1929.
(23) HICK, F. K., R. W. KEETON AND N. GLICKMAN. J. A. S. H. V. E. **10:** 196, 1938.

LOCALIZATION OF THE MEDULLARY RESPIRATORY CENTERS IN THE MONKEY[1]

LINDSAY E. BEATON[2] AND H. W. MAGOUN

From the Institute of Neurology, Northwestern University Medical School

Accepted for publication June 20, 1941

Although it has been accepted that mechanisms for the neural control of respiration are situated in the reticular formation of the medulla oblongata (Finley, 1931; Cordier and Heymans, 1935), only recently have attempts been made, by modern methods, to localize the respiratory region more precisely and to examine the question of its functional subdivision. In 1939 Pitts, Magoun and Ranson, studying responses in the cat to brain stem stimulation with the Horsley-Clarke instrument, outlined a reactive portion of the reticular formation, extending caudally from the level of the facial nucleus over the cephalic four-fifths of the inferior olivary nucleus. Within this area they defined 2 discrete divisions, from which, respectively, coördinated inspiratory and expiratory acts could be elicited. These were designated the inspiratory and expiratory centers. Brookhart (1940), employing slightly different technics and somewhat lower intensities of electrical stimulus, was unable to confirm the existence of these centers in the dog and challenged the concept of functional localization within the respiratory-reactive part of the reticular substance.

The objections raised by Brookhart (1940) to the technical procedures utilized by Pitts, Magoun and Ranson (1939) have been considered by Pitts (1941) and Magoun and Beaton (1941). In addition, in order to study the possibility, implicit in Brookhart's results, of genus and order differences in the organization of central respiratory mechanisms, as well as to examine the medullary regulation of respiration in a form more closely related phylogenetically to man, the present study on the monkey was undertaken.

METHODS. Fourteen monkeys (Macaca mulatta), averaging slightly less than 3 kgm. in body weight, were used. Some were normals; others had been previously subject to acute explorations or chronic lesions of the hypothalamus. No dissimilarities in the respiratory responses could be detected between members of the normal and the operated series. The animals were anesthetized with nembutal, 15 to 25 mgm. per kilogram of

[1] Aided by a grant from the Rockefeller Foundation.

[2] Medical Fellow of the National Research Council.

body weight, injected intravenously and occasionally supplemented with ether during surgical procedures; or with urethane, 0.8 mgm. per kilogram of body weight, injected intraperitoneally. There were no discernible differences of respiratory reaction between the 2 anesthetic groups.

The medulla of each animal was systematically explored by use of the stereotaxic instrument of Horsley and Clarke, after the technic of Ranson (1934). Insertion of the electrodes in the customary vertical plane of the machine was unsatisfactory, because of inability to reach the midline, a difficulty apparently due to lateral deflection of the electrodes by the tentorium cerebelli. Therefore, recourse was had to the posterior electrode carrier, and the medulla was approached from behind. The plane thus afforded is oblique; points on the ventral surface of the brain are 2 mm. further rostral than they would be on a conventional transverse section made at the same dorsal level. However, this amount of inclination is not enough notably to distort the outlines of medullary structures or impede their identification. Operative exposure was achieved by enlarging the foramen magnum with rongeurs as far forward as the transverse sinus and incising the dura mater.

Bipolar electrodes of enameled nichrome wire were used, the exposed tips being separated from one another by 0.2 mm. or less along the axis of the electrodes. The stimulation, that of thyratron regulated condenser discharges, was similar to that used on the cat (Pitts, Magoun and Ranson, 1939; Magoun and Beaton, 1941). The period of excitation was fixed at 15 seconds. A frequency of 300 per second was found suitable, though responses to other frequencies were also analyzed. Intensities of stimulus were varied from 0.9 to 30.0 volts, a strength of 8.7 volts being employed for routine stimulation.

All points stimulated were located on Weil-stained serial sections cut in the plane of the punctures. A series of projection tracings was prepared from sections taken at $\frac{1}{2}$ mm. intervals on the formalin fixed brain, and the stimulated points grouped according to level and plotted on the diagrams. Figure 2 consists of 6 levels selected from this series.

Respiration was recorded as a kymogram by cannulating the trachea, attaching to the cannula a small spirometer built in the form of the familiar basal metabolic rate machine, and fastening a pointer to the moving chamber of the spirometer. Carbon dioxide was absorbed by soda lime in a sleeve placed between the cannula and the spirometer, and oxygen was added as needed. Such a closed system, needing intermittent refilling, yields sloping records; those presented in figure 1 have been trimmed to conserve space.

Observations. As in the cat, many varieties of response were obtained, involving all combinations of changes in amplitude, rate and level of respiration. The concern of this study is with the latter, and only

those reactions showing a definite inspiratory or expiratory tendency have been considered. They have been classified into inspiratory and expiratory

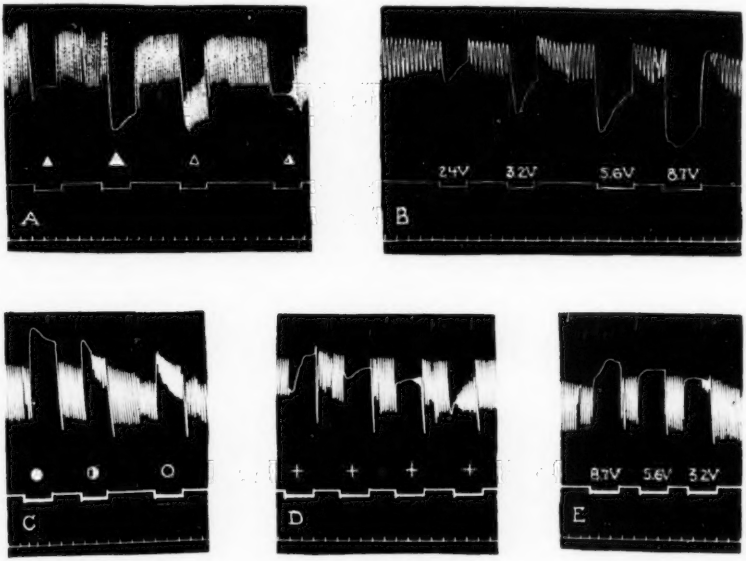


Fig. 1. Spirometer tracings of respiratory reactions to stimulation of the medulla. In A, C and D stimulus strength was 8.7 v. at 300/sec. In all records the time line carries intervals of 6 seconds and the duration of excitation is indicated by the signal magnet. Inspiration is represented by the downstroke, expiration by the upstroke. The symbols accompanying the responses in A, C and D are those used in plotting the localizing diagrams of figure 2. A. Representative inspiratory responses: sustained inspiratory apnea involving an increase in the volume of inspired air of less than 25 cc., a small solid triangle; sustained inspiratory apnea involving an increase of more than 25 cc., a large solid triangle; respiration continued at an augmented inspiratory level, an open triangle; inspiratory apnea breaking into rhythmic respiration before the end of stimulation, a half-filled triangle. B. Increase in the amplitude of inspiratory apnea produced by successively higher voltages administered at a single inspiratory-reactive point. C. Representative expiratory responses: sustained expiratory apnea, a solid circle; expiratory apnea interrupted by periodic respiration during the application of stimulus, a half-filled circle; respiration continued at a heightened expiratory level, an open circle. D. Types of midpositional responses, i.e., apneas midway between the normal expiratory and inspiratory peaks or decreases in amplitude between these levels. E. Decreases in the degree of expiratory response as produced by successive diminution of the strength of stimulus delivered at a representative expiratory-reactive site.

responses of different degrees, midpositional responses and negative responses, as illustrated in figure 1 (A, C and D) and defined in its legend.

The similarity of the reactions in the monkey to those previously described for the cat (Pitts, Magoun and Ranson, 1939; Magoun and Beaton, 1941) renders exhaustive discussion of them unnecessary. The only distinction of any possible moment between the two animals was in the amount of

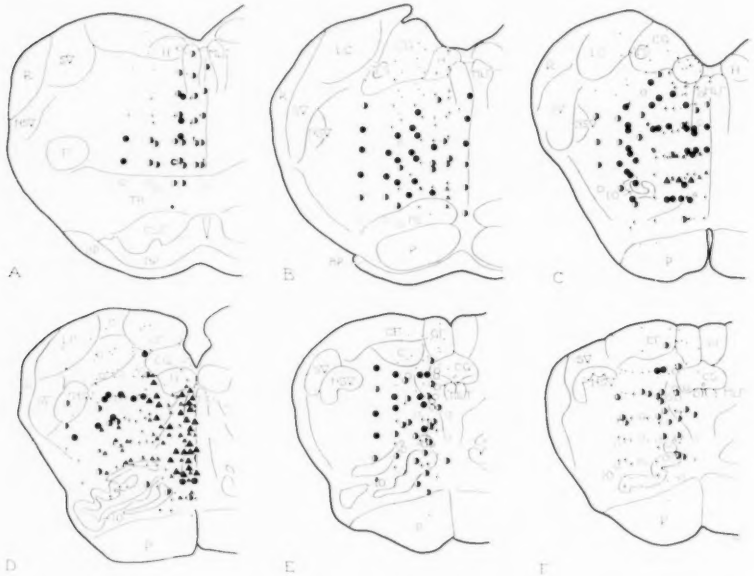


Fig. 2. Six levels through the medulla of the monkey, selected from the complete series, on which have been plotted the responses obtained to 8.7 v. stimulation at 300/sec. Symbols are those explained in figure 1: triangles for inspiratory responses; circles for expiratory; and crosses for midpositional reactions. Negative points are represented as dots. Distances between levels: A–B, 1 mm.; B–C, ½ mm.; C–D, 1 mm.; D–E, ¼ mm.; E–F, ½ mm. Number of animals represented at each level: A, 2; B, 3; C, 3; D, 4; E, 4; F, 4. Brachium pontis, *BP*; cuneate nucleus, *C*; fasciculus cuneatus, *CF*; central grey, *CG*; corticospinal tracts, *CST*; facial nucleus, *F*; fasciculus gracilis, *GF*; hypoglossal nucleus, *H*; inferior olivary complex, *IO*; lateral cuneate nucleus, *LC*; medial lemniscus, *ML*; medial longitudinal fasciculus, *MLF*; nuclei pontis, *NP*; nucleus of the spinal tract of the fifth cranial nerve, *NSV*; pyramid, *P*; restiform body, *R*; spinal tract of the fifth cranial nerve, *SV*; trapezoid body, *TB*; tractus solitarius, *TS*.

air involved. In general, the apneic response to stimulation, especially when it was inspiratory, involved a smaller volume of oxygen in the monkey than in the cat. The reason for this is not clear; rough measurements of lung capacity have not revealed any significant inequalities between the two animals.

As in the cat, 2 separate areas were delineated, from which, respectively, inspiratory and expiratory apneas were consistently elicited. The distribution of responses (to 8.7 volts stimulation) is illustrated in figure 2 on 6 selected sections which cover the entire antero-posterior extent of the respiratory-reactive area. Levels 1 mm. rostral to that of figure 2 A and 1 mm. caudal to that of figure 2 F gave no sustained cessations of respiration.

The inspiratory field (fig. 2 B–D) extends 2 mm. rostro-caudally in the reticular formation of the medulla. Cephalically, slight responses are met with at the very caudal end of the pons, immediately above the medial lemniscus (fig. 2 B). The first maximal inspirations occur $\frac{1}{2}$ mm. analward where they are found lying medially to the rostral extremity of the inferior olive (fig. 2 C). Proceeding caudad, the inspiratory region rapidly enlarges both dorsally and laterally and at the level of figure 2 D reaches the midline and the dorsal limits of the reticular formation. At this level sustained inspirations are obtained from a field reaching from a point between the 2 inferior olives to one immediately beneath the hypoglossal nucleus and spreading laterally some 3 mm. from the midline in the area dorsal to the olive. A level (not illustrated) $\frac{1}{2}$ mm. caudal to that represented by figure 2 D demonstrates a similar distribution of responses except that the lateral reach of the center is decreased to 2 mm. One millimeter posterior to level D, inspiratory responses abruptly disappear (fig. 2 E). Thus the inspiratory area lies dorsally and medially to the rostral half of the inferior olivary nucleus in a field which at its rostral end is basally and medially located and which, toward its caudal end, gradually expands both dorsally and laterally.

The expiratory field (fig. 2 A–F) surrounds the inspiratory, lying rostrally, laterally and caudally to the latter, and also dorsally to it except at that level where the inspiratory area reaches the hypoglossal nucleus (fig. 2 D). In addition, scattered expiratory reactions can be elicited from sites beneath the inspiratory center (fig. 2 C and D). Maximal expiratory apneas are obtained from the midline only ahead of the inspiratory region (fig. 2 B and C), and caudal to the latter area expiratory responses are generally weak and dispersed (fig. 2 E and F). The greatest lateral extent of the expiratory center is 4 mm. from the median raphé. For purposes of rough description, the expiratory field can be said to be coextensive with the reticular formation from a level slightly more than 1 mm. ahead of the rostral end of the inferior olive to the latter's caudal extremity, except for the compact area occupied by the inspiratory center. On the whole expiratory responses are more disseminated than are inspiratory responses.

The relation of the respiratory fields to familiar dorsal landmarks of the brain stem is shown in figure 3, which is a projection of those fields,

as reconstructed from the complete set of levels, onto the floor of the 4th ventricle. The regions outlined are those giving sustained responses to 8.7 volts stimulation. The projection was made, not in the plane of the sections of figure 2, but in the true transverse plane.

Close inspection of figure 2 reveals a suggestive apportionment of what are characterized as "midpositional points," i.e., points yielding apnea anywhere between normal expiratory and inspiratory levels or a reduction of respiratory excursion between these limits. These midpositional responses (plotted as crosses), though somewhat sporadic in distribution, are clumped in 2 particular situations. First, they are found skirting the

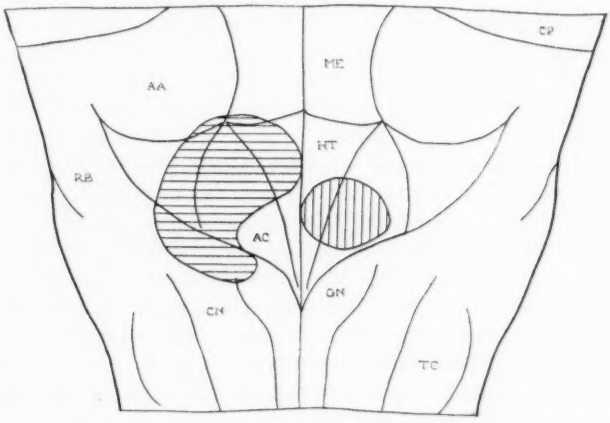


Fig. 3. Dorsal view of the lower brain stem of Macaca mulatta with cerebellum removed. The areas of sustained inspiratory apneas (on the right; vertical lining) and sustained expiratory apneas (on the left; horizontal lining) are shown projected onto the floor of the 4th ventricle. To avoid overlapping the two regions are shown on different sides of the brain stem. Area acoustica, *AA*; ala cinerea, *AC*; cuneate tubercle, *CN*; cerebellar peduncle, *CP*; clava, *GN*; hypoglossal trigone, *HT*; medial eminence, *ME*; restiform body, *RB*; tuberculum cinereum, *TC*.

outer boundary of the expiratory field (fig. 2 B, C and F), (they were elicited rostrally and caudally to the expiratory area at levels not illustrated). In this locus these were usually midpositional apneas tending toward the expiratory side and were mingled with expirations which broke into rhythmic respiration and continued respirations of increased expiratory height. In these 3 types of reaction the points stimulated may not have been sufficiently close to the center of concentration of reactive elements to produce sustained responses. The second location of midpositional points is a zone between the inspiratory and expiratory fields (fig. 2 C and D). Here such responses were midpositional apneas, some

of which moved during the period of stimulation toward the expiratory side of the kymograph tracing, some toward the inspiratory. This band, intermediate between the inspiratory and expiratory centers, may represent a situation in which roughly equal numbers of inspiratory and expiratory elements are subjected to excitation. The midpositional reactions of figure 1 D were obtained as the electrode passed from an expiratory region to an inspiratory, and the shift of the level of respiration from the expiratory to the inspiratory side is clearly evident.

In general, stimulation of medullary structures other than the reticular substance did not yield the respiratory responses in question. When they were obtained from other formations, it was from those directly contiguous with the main reactive fields. These aberrant points were diminished in number when stimulus strength was reduced and they are presumably due to current spread to the more specifically sensitive areas.

Investigation of changes in the types and localization of responses with alteration in stimulus strength has not been a primary aim of this study. So that the topography in the monkey might be compared with that delineated for the cat (Pitts, Magoun and Ranson, 1939), routine stimulation was done at 8.7 volts. However, many experiments were done with lower stimulus strengths (0.9 to 5.6 volts); the results confirmed the conclusions drawn from the investigation of low voltage responses in the cat (Magoun and Beaton, 1941). In addition, reactions to a higher voltage (13.7 volts) were examined. Reduction of the intensity of excitation caused some shrinkage of the respiratory fields as well as some diminution in the magnitude of responses, especially those responses found at the peripheries of the fields. Yet, many sustained inspirations were obtained at 2.4 volts, some at 0.9 volts. Sustained expirations were procured by voltages down through 3.2 volts. The topography of response was in no way changed by the use of lower voltages, and in no instance was the direction of response altered by a voltage decrement or, for that matter, by a frequency change. Figure 1 (B and E) illustrates representative alterations in the magnitude of inspirations and expirations with a succession of voltages. It also shows that the volume of inspiratory apnea was usually greater than that of expiratory apnea. These findings, even to the threshold voltage values, are in striking concordance with results in the cat (Magoun and Beaton, 1941).

Two macaques were subjected to medullary stimulation 2 weeks after a left hemisection at the pontile level. No significant differences were found between responses elicited at the same locations on the 2 sides of the brain, and almost maximal inspirations and expirations were obtained from both sides in each animal.

Discussion. The localization of inspiratory and expiratory fields has been found to be as precise in the monkey as in the cat. The delimitation

of these fields by the use of "high voltages" (8.7 volts) is deemed entirely permissible because of evidence elsewhere presented (Pitts, 1941; Magoun and Beaton, 1941). By having outlined these areas with relatively high stimulus strengths, any error would seem to be in the direction of diffusion, especially since employment of lower voltages has given some contraction of the regions with maintenance of their general topography and relationships.

The anatomical locations of the respiratory centers in the monkey correspond in general with those of the cat. The topographical differences in the two animals do not seem too great to be explained by very evident variance of medullary structures in these members of separate taxonomic orders. The inspiratory region is less and the expiratory region more disperse in the monkey than in the cat. The inspiratory area of the cat lies ventrally to the expiratory throughout the extent of their anteroposterior overlap, while in the monkey the inspiratory field, at its caudal extremity, rises high in the midline and no expiratory responses are obtained dorsally to it. Also, in the cat, expirations cannot be elicited from loci caudal to the inspiratory center as they can in the monkey. Some of these dissimilarities in the topography of the excitable regions in the two animals may perhaps be related to the larger size of the inferior olive in the monkey and the consequent alteration in the shape of the reticular substance.

The cephalic and caudal boundaries defined for the respiratory area in the monkey agree with the limits set for the dog by tapping from the brain stem amplified potentials showing a respiratory rhythm (Gesell, Bricker and Magee, 1936). The inability to demonstrate discrete expiratory and inspiratory centers in the dog (Brookhart, 1940) contrasts with the success now achieved in both the cat and monkey.

A comparison of the location of the respiratory centers in monkey and man cannot be made with any precision, due to the lack of exact information on the site of the centers in man. A rough accordance is indicated by reported cases of clinical neurogenic respiratory failure (Finley, 1931; Nordmann and Müller, 1932) in which the lesions were in the reticular formation overlying the inferior olive.

The finding that left pontile hemisection in 2 animals did not affect the bilateral distribution or magnitude of response is regarded as a further contribution to other evidence at hand (Magoun and Beaton, 1941) that the respiratory reactions elicited by medullary stimulation should probably not be attributed to the excitation of clustered direct afferents to the reticular formation.

SUMMARY

Circumscribed electrical stimulation of the medullas of 14 monkeys by means of the Horsley-Clarke technic has revealed the existence of 2 dis-

crete regions, from one of which sustained inspiratory apnea, from the other sustained expiratory apnea were consistently obtained. The inspiratory field is located dorsally and medially to the rostral half of the inferior olive. The expiratory field surrounds the inspiratory, lying rostrally, caudally, laterally and to some extent dorsally to the latter. Successive decreases in stimulus strength down to threshold values did not change the character or topographical arrangement of the responses. The anatomical localization is in general agreement with that found previously for the cat. In 2 monkeys with pontile hemisections, there was no diminution of reaction as compared with normal animals nor any difference in the responses obtained from the 2 sides of the medulla.

REFERENCES

Brookhart, J. M. This Journal **129:** 709, 1940.

Cordier, D. and C. Heymans. Ann. de physiol. **11:** 535, 1935.

Finley, K. H. Arch. Neurol. Psychiat. **26:** 754, 1931.

Gesell, R., J. Bricker and C. Magee. This Journal **117:** 423, 1936.

Magoun, H. W. and L. E. Beaton. This Journal **134:** 186, 1941.

Nordmann, M. and O. Müller. Klin. Wchnschr. **11:** 1371, 1932.

Pitts, R. F. This Journal **134:** 192, 1941.

Pitts, R. F., H. W. Magoun and S. W. Ranson. This Journal **126:** 673, 1939.

Ranson, S. W. Psychiat. en neurol. bl. **38:** 534, 1934.

RESPIRATORY RESPONSES FROM STIMULATION OF THE MEDULLA OF THE CAT[1]

H. W. MAGOUN AND LINDSAY E. BEATON[2]

From the Institute of Neurology, Northwestern University Medical School

Accepted for publication June 30, 1941

In a study of the respiratory responses to stimulation of the medulla of the cat, Pitts, Magoun and Ranson (1939) described an excitable reticular field from whose dorsal and ventral parts expiratory and inspiratory reactions were respectively elicited. The results appeared to extend our knowledge of the location and functional organization of the respiratory center, but experiments on the dog led Brookhart (1940) to conclude that the respiratory responses described could be obtained only by high voltage stimulation whose great range of spread prevented localization of reactive regions. Emphasis has recently been placed by Gesell (1940) upon an alternative explanation of the responses from medullary stimulation, i.e., that they result from activation of afferent fibers exerting a predominantly inspiratory or expiratory influence upon respiration.

It seemed desirable, therefore, to review the respiratory responses from the medulla of the cat with reference to the voltage and range of spread of stimulating current and the distribution of reactive areas. In addition responses have been examined after chronic ablation procedures designed to eliminate certain afferent pathways whose excitation might be responsible for the responses.

METHODS. In lightly anesthetized cats, respiration was recorded with a spirometer and stimulation or the production of lesions was carried out with the Horsley-Clarke technic (Ranson, 1934). Unless noted, stimuli consisted of thyratron regulated condenser discharges at 300 per second with voltages between 0.9 and 8.7.

RESULTS. *Respiratory responses to stimulation.* The responses to which attention was directed are shown in figure 1; at the left are a group of expiratory responses elicited from the dorsal reticular formation and at the right are a group of inspiratory responses obtained from the ventral reticular formation. In each group, on increasing the voltage of stimulation, the increased magnitude of reaction is apparent both in the extent

[1] Aided by a grant from the Rockefeller Foundation.

[2] Medical Fellow of the National Research Council.

to which the response dominates over regular breathing and in addition by the increased amplitude of expiration or inspiration.

Distribution of reactive areas. Data on the distribution of excitable areas for these respiratory responses on altering the voltage of stimulating current have been obtained by activating a series of points in transverse levels through the excitable region, and at each point recording the responses to voltages between 0.9 and 7.4. This range was chosen because results obtained with 1 volt were those to which Brookhart (1940) attached significance, while stimuli of 8 volts were used routinely by Pitts, Magoun and Ranson (1939).

The results of an experiment are shown in figure 2. A series of points were stimulated in levels I and II through the excitable region and on

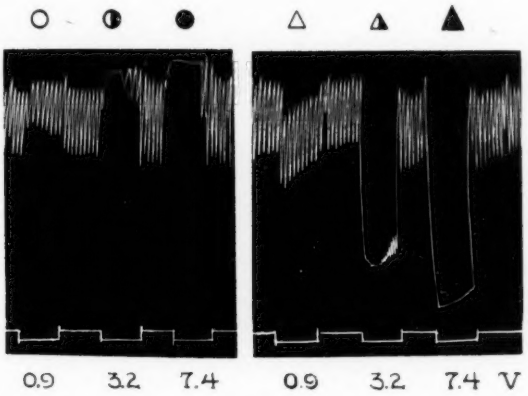


Fig. 1. Spirometer tracings showing weak, intermediate and marked expiratory (left) and inspiratory (right) responses to 15 seconds' stimuli at the voltages indicated. The symbols above designate the type of response in figure 2.

3 copies of each level are shown the site and magnitude of reactions to voltages of 0.9, 3.2 and 7.4. An examination of the data presented in the figure reveals that in the case of both the expiratory and the inspiratory fields the effects of increasing the intensity of stimulation, within the range of 1 to 8 volts, is primarily to augment the magnitude of the responses obtained and not appreciably to increase the distribution of the reactive areas. The excitable reticular formation encountered by Pitts, Magoun and Ranson (1939) is evident with each of the voltages used and its subdivision into dorsal expiratory and ventral inspiratory zones is clearly apparent.

Threshold. A number of observations, some of which are indicated in figure 2, permit the conclusion that with thyratron regulated discharges

at 300 per second, the threshold for marked inspiratory responses is from 1 to 2 volts, that for marked expiratory reactions is from 3 to 4 volts. Similar low thresholds were found using 60 per second sine wave stimuli.

Effect of lesions at the point of stimulation. The repetition of stimuli after producing lesions around the point of stimulation is a method used by Brookhart (1940) to estimate the distance of current spread. The

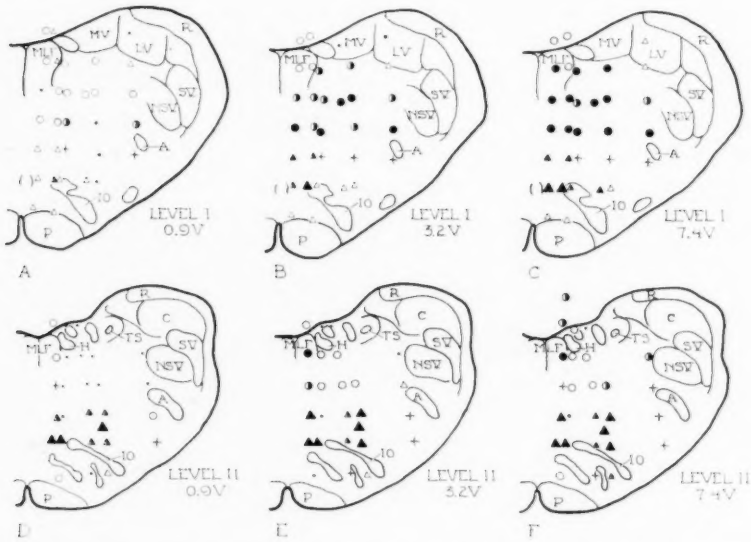


Fig. 2. Transverse sections of the medulla of the cat, showing the respiratory responses obtained at 2 levels of stimulation with voltages of 0.9, 3.2 and 7.4. The results from both halves of the medulla are shown on the right side. Expiratory responses are indicated by circles and inspiratory reactions by triangles as in figure 1. Small and large filled triangles indicate marked inspiratory responses of less and more than 75 cc., respectively. Crosses show mid-positional changes, and dots, negative points. Abbreviations are as follows: nucleus ambiguus, *A*; lateral cuneate nucleus, *C*; hypoglossal nucleus, *H*; inferior olive, *IO*; lateral vestibular nucleus, *LV*; medial longitudinal fasciculus, *MLF*; medial vestibular nucleus, *MV*; nucleus of spinal fifth tract, *NSV*; pyramid, *P*; restiform body, *R*; spinal fifth tract, *SV*; tractus solitarius, *TS*.

effects of a number of lesions were studied in the present investigation and the results of an experiment are shown in figure 3.

An electrode was inserted into the ventral reticular formation and the inspiratory responses to stimuli between 0.9 and 8.7 volts were recorded (fig. 3 A). After the production of a lesion, the same voltage series of stimuli was repeated and the responses obtained in the first minutes were

either abolished or greatly reduced (fig. 3 B). Improvement occurred and the responses obtained 18 and 32 minutes after the lesion reflected the development of a rather steady state (fig. 3, C, D). The final response elicited with 8.7 volts (fig. 3 D) was equal in magnitude to that obtained initially with 2.4 volts (fig. 3 A), amounting to an over-inspiration of 60 cc.

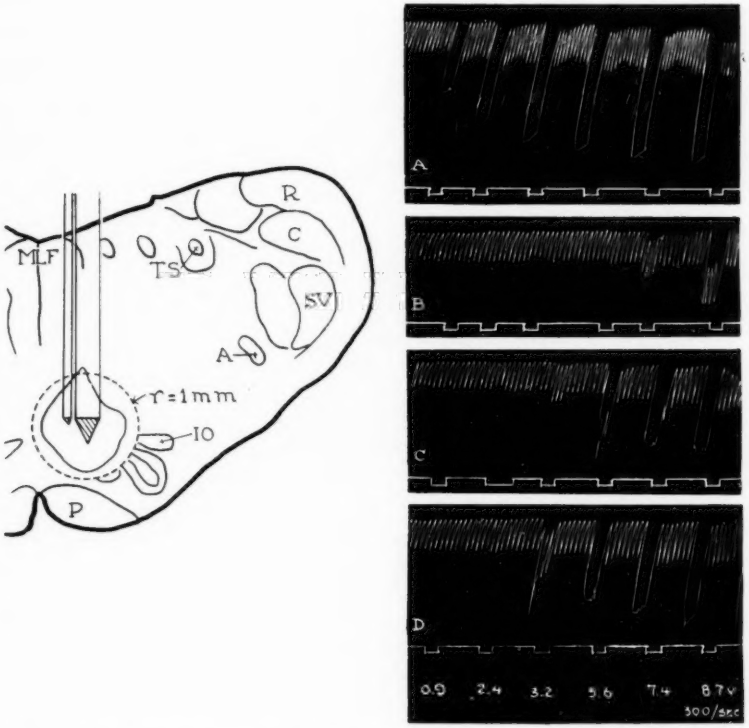


Fig. 3. Transverse section of the medulla (left), showing the position of electrodes and lesion. The dotted circle inclosing the lesion has a radius of 1 mm. drawn to scale. At the right are spirometer tracings of inspiratory responses obtained before (A), and 1 minute (B), 18 minutes (C), and 32 minutes (D) after the lesion. The voltages of the 15 second stimuli are indicated.

Since the response obtained initially with 2.4 volts, unlike those to higher voltages, remained abolished after the lesion, it was presumably elicited entirely from the volume of tissue destroyed, which extended in every direction some 0.5 mm. from the surface of the electrodes. The final equivalent response obtained with 8.7 volts would have been elicited

from an equal volume of tissue if a linear relation existed between the magnitude of response and the amount of tissue activated. This tissue if distributed evenly around the margins of the lesion would extend some 0.7 mm. from the surface of the electrodes, and according to this reasoning a stimulus of 8.7 volts should spread about 0.7 mm.

Infrequently a transient augmentation of response was found in the early period after producing a lesion. Because the magnitude of reaction does not vary greatly when stimuli are repeated at intervals over a long period, a sudden increase sometimes found after making a lesion (see also Brookhart, 1940) suggests that some factor associated with the lesion has increased the usual range of current spread or augmented the excitability of the surrounding tissue. Such a suggested alteration may regularly be present and the responses obtained after lesions may reflect the balance struck between it and response reducing factors. In our opinion further study of the lesion method should be undertaken before using it as a simple test of current spread.

Partial deafferentation. Attention may here be directed away from questions of method to a consideration of the possibility that the responses obtained from medullary stimulation (fig. 1) are the result of activation of afferent fibers exerting an influence upon respiration. The results of Pitts, Magoun and Ranson (1939) showed that these responses were not elicited from the nucleus of the tractus solitarius or the posterior column nuclei where they might have been expected to be obtained, were excitation of glossopharyngeal, vagal or thoracic proprioceptive afferents the basis of their production.

To investigate the possibility that unknown afferent pathways from the vagus and glossopharyngeal nerves to the reticular formation were responsible for the results, the distribution, magnitude and threshold of responses from the 2 halves of the medulla were compared 2 weeks after intracranial section of the glossopharyngeal and vagal rootlets on 1 side. With minor variations, which were without regard as to laterality, all features of the reactions on the 2 sides showed close similarity.

In each of 2 other cats, the respiratory responses from the 2 halves of the medulla were compared after chronic hemisection of the spinal cord at C 1. Exploration of 4 levels was completed and in one level a slight restriction of the inspiratory field on the side of hemisection was noted and the amplitudes of both expiratory and inspiratory responses were smaller on this side. In the other 3 levels no significant difference in the features of the responses from the 2 halves of the medulla could be observed.

These results, which should be combined with those obtained after chronic pontile hemisection in the monkey (Beaton and Magoun, 1941), permit the statement that the responses to medullary stimulation are not

attributable to the activation of a direct, ipsilateral, afferent pathway approaching the reticular formation either from the pons, or from the spinal cord, or by way of the vagus or glossopharyngeal nerves.

SUMMARY

The respiratory responses obtained from stimulation of the medulla of the cat have been reviewed with reference to the voltage and range of spread of stimulating current and the distribution of reactive areas. The results do not support the view that these responses are dependent upon the activation of large, indiscriminately situated regions of the medulla by widely spreading stimuli, but indicate, on the contrary, definite areas responsive to locally acting excitation.

REFERENCES

BEATON, L. AND H. W. MAGOUN. This Journal **134:** 177, 1941.
BROOKHART, J. M. This Journal **129:** 709, 1940.
GESELL, R. Ergebn. Physiol. B. **43:** 570, 1940.
PITTS, R. F., H. W. MAGOUN AND S. W. RANSON. This Journal **126:** 673, 1939.
RANSON, S. W. Psychiat. en neurol. bl. **38:** 534, 1934.

THE DIFFERENTIATION OF RESPIRATORY CENTERS[1]

ROBERT F. PITTS[2]

From the Department of Physiology, New York University College of Medicine, New York City

Accepted for publication June 23, 1941

While it is generally agreed that the basic neural elements necessary for integration and regulation of respiration are to be found within the reticular formation of the medulla oblongata caudal to the level of entrance of the eighth nerves (Cordier and Heymans, 1935), no concept of the morphological organization of this so-called respiratory center is universally accepted. Gesell, Bricker and Magee (1936) studying action potentials tapped from the lower brain stem, and Brookhart (1940) stimulating the same region with shocks of near threshold intensity, conclude that the neurones controlling inspiratory and expiratory activities are diffusely intermingled and show no grouping into discrete centers. On the other hand Nicholson (1936) observing respiratory modifications to local cooling of the floor of the fourth ventricle, and Pitts, Magoun and Ranson (1939a) stimulating the brainstem with shocks of moderate intensity, conclude that the respiratory center may be divided into a dorsal expiratory and a ventral inspiratory center. The latter investigators localized the inspiratory center to the ventral reticular formation overlying the cephalic four-fifths of the inferior olivary nucleus, and the expiratory center to the dorsal reticular formation, dorsal to, slightly cephalic to, and cupped over the cephalic end of the inspiratory center.

Brookhart (1940) and Gesell (1940) have criticized this localization along three lines: 1, excessive physical spread of stimulating current; 2, with such spread a functionally meaningless differentiation of the respiratory center into inspiratory and expiratory divisions; 3, too all inclusive criteria for differentiation of centers which might mask simultaneous activation of antagonistic elements.

Although various indirect lines of evidence could be brought forward in answer to the above criticisms, it was felt that the importance of accurate knowledge of the morphology of the respiratory center justified a reinvestigation of the problem of differentiation. Accordingly experiments were

[1] Aided by a grant from the Penrose Fund of the American Philosophical Society.

[2] Littauer Fellow of Neurophysiology.

designed to provide more or less direct information on each of the points in question.

The measure of stimulus spread. Methods proposed to assess physical spread of stimulating current within the brain have been based either upon the distance the electrode tips must be advanced to abolish or reverse a motor response (Pitts, Magoun and Ranson, 1939a; Hinsey, 1940), or upon the size of an electrolytic lesion necessary to abolish the response at a given placement of the electrodes (Brookhart, 1940). These methods give only qualitative results; the latter, in addition, is complicated by a marked change in physical properties of the tissue surrounding the lesion (Magoun and Beaton, 1941).

The experimental preparation used to provide more quantitative data on the problem of current spread within the brainstem of the cat is illustrated in diagrammatic form in figure 1. Fibers in the sensory frontal branch of the trigeminal nerve have their cell bodies in the Gasserian ganglion, enter the brain at a pontile level, and turn sharply caudad in the bulbar trigeminal tract, located laterally throughout the medulla. Collaterals are given off in the nucleus and after synapsis the secondary trigeminal pathways continue. If recording electrodes are placed on the frontal nerve this primary sensory system may be activated in reverse by stimuli applied to the tract through bipolar needle electrodes oriented in the Horsley-Clarke stereotaxic instrument.[3] Stimuli are effective only when they are applied to the tract itself or to the collaterals as they pass into the nucleus of the tract located just medially. Activation of the secondary pathways leads to conduction only to the cell body in the nucleus, since excitation does not pass the synapse the wrong way.

Stimuli applied to the tract were brief, thyratron regulated condenser discharges (time constant approximately 0.1 m. sec.) at an intensity of 8 volts[4] and at a frequency of 100 per second. A bridge transformer between stimulator and electrodes reduced stimulus artifact. Potentials of the frontal nerve were amplified by a condenser coupled amplifier and applied to a cathode ray oscillograph. The cathode ray sweep was synchronized so as to produce a standing wave which could be measured or photographed. The height of the potential record serves as a rough indication of the number of fibers which lay within a zone about the electrode tips where the stimulus intensity was above threshold. On the left of figure 2 is a projected tracing of a Weil stained section of the cat brain showing the position of the needle electrode. The interruptions of the black line indicate the

[3] The type of needle electrode used and the method of histological identification of structures stimulated were described in our original communication (Pitts, Magoun and Ranson, 1939a).

[4] Repeated checks of voltage at the electrodes were made during each experiment by switching directly from electrode terminals to oscillograph.

position of the needle tip at each half millimeter as it was lowered into the medulla. On the right are records of the potentials evoked in the frontal nerve. No response was obtained until position 1 was reached where a minute deflection occurred. The deflection which occurs in all records at

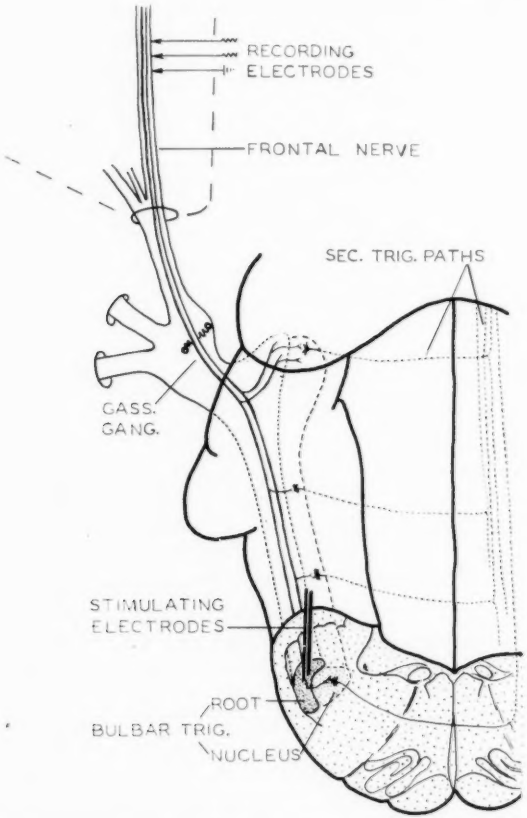


Fig. 1. Diagrammatic three-dimensional sketch of the lower brainstem of the cat with cerebellum removed, to illustrate the use of the frontal-nerve-bulbar-tract system to assess stimulus spread; see text for details.

the beginning of the sweep is stimulus artifact. Lowering the electrode ½ mm. from position 1 to 2 increased the response tremendously. Penetration of the needle tip was stopped at this point in order to mark the position accurately.

A similar experiment is shown in figure 3 except that two intensities

of stimuli were applied at each level of electrode penetration, namely, 8 and 16 volts. No response was obtained until the electrode tips penetrated

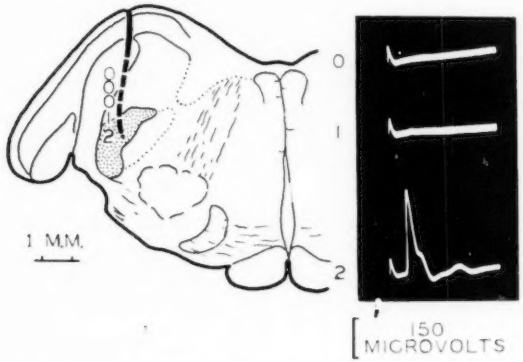


Fig. 2. Potentials recorded from the frontal nerve on lowering of the stimulating electrode in successive half-millimeter steps into the bulbar trigeminal root (stippled). Stimulus, 8 volts; initial deflection of the sweep, stimulus artifact. Position of electrode tips at each level corresponding to potential record is shown by the break in the heavy black line representing the track of the electrode.

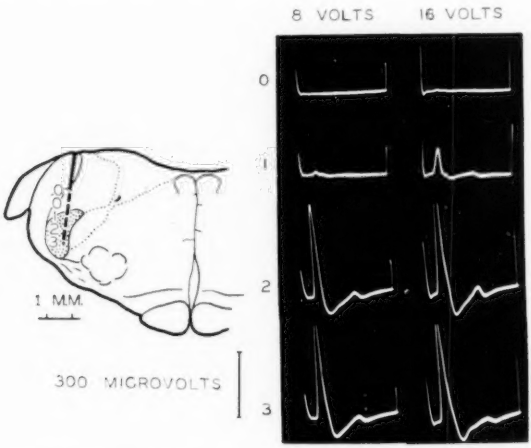


Fig. 3. Potentials recorded from the frontal nerve during stimulation at each half-millimeter level approaching and penetrating the bulbar trigeminal root with stimuli of two intensities, 8 and 16 volts. At position 3 the threshold was slightly less than 1 volt.

the tract and at position 1 (8 volts) a just perceptible response was evident. Increasing the stimulus to 16 volts increased the evoked potential some-

what, no doubt the result of increased current spread and consequent activation of more fibers within the tract. But lowering the electrode $\frac{1}{2}$ mm. at 8 volts produced a much greater increase in response than doubling the voltage at the previous position. At position 3 an even larger response was obtained both at 8 and 16 volts. At this position the intensity of the stimulus was lowered until a just perceptible response was obtained. The threshold was slightly below 1 volt. It is quite apparent then that stimulus intensity diminishes rapidly with distance from the electrode tips, falling to a value of about one-eighth or less a half millimeter away.

The measure of degree of localization possible. It may be concluded from the preceding results that at least theoretically the use of brief condenser shocks at an intensity of 8 volts should permit localization of structures within the brainstem with an error not greater than one-half millimeter,

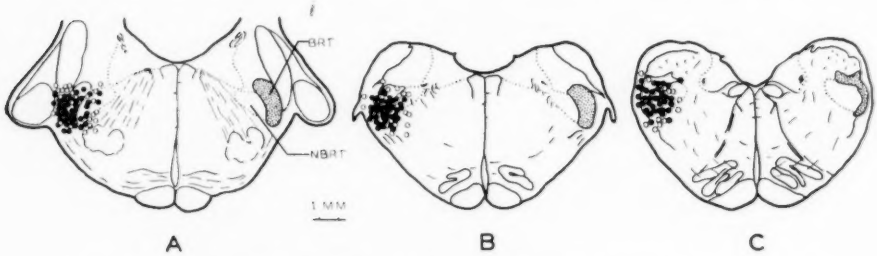


Fig. 4. Comparison of the bulbar trigeminal root as localized by stimulation (left) with its known morphology (right). Solid circles, frontal nerve potentials from one quarter to the maximum obtained in any given experiment; open circles, frontal nerve potentials of lesser magnitude. The dotted enclosure medial to the stippled root represents the nucleus of the bulbar trigeminal root.

possibly less. However, the bulbar-tract-frontal-nerve preparation is admirably suited for a direct comparison of physiological localization with known morphology, for the limits of the tract are fairly well defined.

A total of 10 experiments were performed exploring the medulla from the midline outward at a number of levels, with a stimulus intensity of 8 volts. The height of the frontal nerve response was measured on the face of the cathode ray tube at each position of the needle electrode. On the left side of the sections in figure 4 is shown the physiological localization of the bulbar trigeminal tract by the stimulation technique. On the right side in stippling is shown the true morphology of the tract. The dotted enclosure placed just medial to the tract represents the nucleus of the bulbar tract in which one would expect some collaterals from frontal nerve fibers. The solid circles in figure 4 represent evoked potentials of one-quarter or more of the maximum obtained in a given experiment, while the

open circles represent potentials of lesser magnitude. The least potential discernible in the frontal nerve at the amplification used amounted to about 0.5 per cent of the average maximum.

While the physiological localization is not a photographic likeness of the bulbar tract, it misses the boundaries on an average less than $\frac{1}{2}$ mm., except medially, where collaterals pass into the bulbar nucleus. To this extent the physiological localization of the course of the primary trigeminal sensory axon is a more accurate one than that provided by inspection of a low power projection of the tract for the terminations of these axons in the nucleus are not apparent except at higher magnifications.

Although the original experiments localizing inspiratory and expiratory divisions of the respiratory center (Pitts, Magoun and Ranson, 1939a) had been performed with the same intensity and essentially the same wave form of stimulus as above, it was felt desirable to repeat them under exactly the same conditions. Hence a stimulus of 8 volts was applied through the

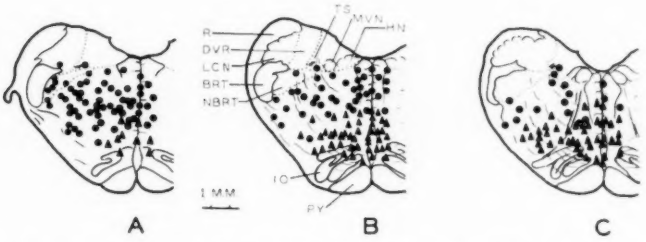


Fig. 5. Sections through the medulla oblongata of the cat at millimeter intervals to show the localization of the maximal inspiratory responses (triangles) and maximal expiratory responses (circles) described in the text.

same bridge transformer and the respiratory response measured by a closed spirometer circuit identical to that employed in the original investigation. A frequency of 240 per second was employed. The medulla was explored millimeter by millimeter within the general confines previously described for the center. Only the maximal responses are considered, namely, inspirations of 75 cc. or over plotted in figure 5 as triangles and expirations as great or greater than normal plotted as circles. Only inspirations and expirations maintained without interruption by rhythmic respiration for the duration of stimulation (12 sec.) were considered maximal.

The maximal respiratory responses shown in figure 5 represent the composite plot of results obtained on 6 cats at each level. Only the rostral 3 mm. of the respiratory center are shown. The dorsal distribution of expiratory responses and the ventral distribution of inspiratory ones are sufficiently obvious to require no further comment. A comparison of

sections A, B and C with sections C, D and E of figure 3 (Pitts, Magoun and Ranson, 1939a, p. 679) shows a remarkably close agreement. The only difference noted in this reinvestigation worth commenting upon is a more caudal extent of expiratory responses than was described previously, these reponses extending approximately as far caudally as the inspiratory ones, i.e., some 2 mm. caudal to section C of figure 5.

A consideration of the results presented in the preceding two sections leads to the conclusion that the stimulus is of threshold intensity only within a radius of one-half millimeter around the electrode tips; that in actual practice a structure may be localized within the brain to within at least one-half millimeter; and that the dorsal position of expiratory and ventral position of inspiratory responses must have a morphological basis.

Criteria for differentiation of the divisions of the respiratory center. Pitts, Magoun and Ranson (1939b) summarized the evidence that the maximal inspiratory and expiratory responses result from activation of two antagonistic and morphologically distinct divisions of the respiratory center, and these points need not be repeated here. It was felt that further evidence might be obtained by a study of the behavior of single respiratory motorneurones during stimulation of these centers, especially as concerns the possibility of simultaneous activation of excitatory and inhibitory reticular elements.

Accordingly, small strands containing one or more active fibers were carefully teased from the cut third phrenic root of the cat and potentials recorded by a condenser-coupled amplifier. Simultaneous respiratory tracings were obtained by connecting the tracheal cannula through a soda-lime tube to a 5-gallon bottle filled with oxygen. The small pressure changes of the closed system were measured by a light rubber optical tambour.

Figure 6 shows the potential records obtained from a small slip of the phrenic nerve in which, during the control strip of record, only a single fiber was spontaneously active, firing four impulses per inspiration. As the stimulating electrode was progressively lowered a millimeter at a time, through the dorsal and into the ventral reticular formation, stimulating at each level, records A, B, C and D were obtained. In records A and B obtained on stimulation of the dorsal reticular formation, the last of the four expected nerve impulses was clipped off and expiratory apnea maintained, for the duration of the stimulus. A millimeter shift of the electrode into the ventral reticular formation (B to C) altered the response from one of inhibition to excitation and in records C and D not only is the neurone of the control record active but at least 3 others as well. The position of the electrode at each level of the stimulation is shown in figure 7. Again, confirmation of the expiratory, or as concerns the phrenic, the inspiratory-inhibitory function of the dorsal reticular formation, and the inspiratory

function of the ventral reticular formation, is evident. While inhibition of activity similar to that shown in A and B of figure 6 may be obtained by central stimulation of the vagus, the expiratory reactive area outlined in figure 5 does not conform with the course of primary sensory vagal neurones. That secondary sensory vagal fibers enter the dorsal reticular formation and there establish tertiary or higher order connections is highly probable. On the other hand, stimulation of the ventral reticular forma-

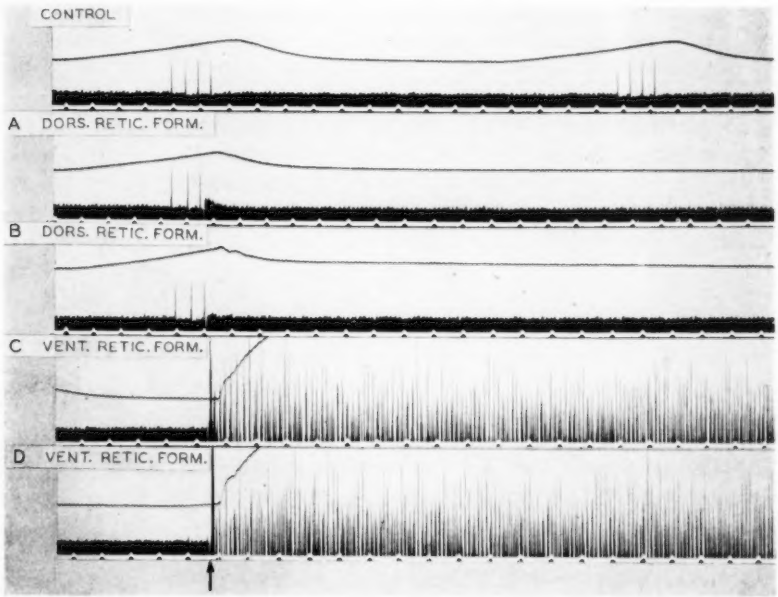


Fig. 6. Potentials recorded from a small slip of the phrenic nerve during stimulation of the medulla at the levels shown in figure 7. The time of application of the stimulus in records A to D is indicated by the arrow at the bottom of the figure. Stimulus, 8 volts, 240 per second; time, one-fifth second; upstroke of respiratory record indicates inspiration.

tion leads to a degree of excitation of phrenic neurones maintained for minutes which in our experience can be obtained from no afferent nerve. This of course does not mean that no elements pass through the dorsal reticular formation which have an excitatory effect. Indeed, second order vagal afferents, excitatory in nature, must send their processes across the dorsal to reach the ventral reticular formation. It merely means that the preponderance of elements of the dorsal reticular formation are inhibitory so far as the motor outflow of the phrenic is concerned, and that the pre-

ponderance of the ventral reticular elements are excitatory. Furthermore a localized stimulus, involving a millimeter cube of either the dorsal or ventral reticular formation, is capable of dominating the entire extent of that formation and inhibiting completely activity in the antagonist.

Discussion. The rather extensive control experiments cited provide a needed direct means of assessing current spread and degree of localization possible within the brainstem. They have shown that the field produced about the tips of bipolar needle electrodes by brief repetitive condenser discharges diminishes rapidly in intensity to one-eighth or less at a distance of $\frac{1}{2}$ mm. Utilizing such a method for physiological exploration, a well defined structure such as the bulbar trigeminal tract may be localized to within $\frac{1}{2}$ mm.

When this method was applied to the localization of the maximal respiratory responses described by Pitts, Magoun and Ranson (1939a), the original localization was confirmed in all essential details. One is therefore forced to conclude some morphological difference between the dorsal and ventral

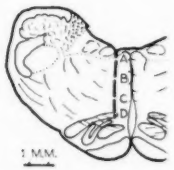


Fig. 7. Locus of the points stimulated in records A to D of figure 6. Note that A and B fall within the region defined as the expiratory center while C and D are in the inspiratory center.

reticular formations to account for the respective localizations of expiratory and inspiratory responses.

Stimulation of the ventral inspiratory center leads to repetitive activity of phrenic motor neurones, recruiting in new units not spontaneously active. Activation of the dorsal expiratory center, on the other hand, stops the spontaneous motor discharge of the phrenic. These results have been repeatedly confirmed and provide confirmatory evidence, not only for the dorso-ventral distribution of expiratory and inspiratory elements, but also for the localized character of the stimulus. If a level is stimulated in the region of junction of dorsal and ventral reticular formations (e.g., halfway between B and C of fig. 7), some admixture of inhibitory and stimulating effects is observed as might be expected.

The contrary results of Brookhart (1940) seem explicable in part on the near threshold intensity of stimulation which he used in the majority of his experiments. The failure to obtain a type of localization of maximal respiratory responses in the dog similar to that in the cat in the few ex-

periments in which higher stimulus intensities were employed, is unexplained. That it is due to a species difference between cat and dog is improbable in the light of the results of Beaton and Magoun (1941) in the monkey. Furthermore, the experiments of Nicholson (1936) on the dog are most readily explained on the basis of a morphological dissociation of expiratory and inspiratory centers much as found in the cat. The results of Gesell, Bricker and Magee (1936), though interesting, scarcely bear on the question of differentiation between inspiratory and expiratory motor centers. The potentials which they recorded from such afferent structures as the posterior columns, gracile and cuneate nuclei, internal arcuate fibers, tractus solitarius, lateral reticular nucleus, etc., obviously have their origin within proprioceptive end organs responsive to the respiratory movements of the animal. These structures, distributed widely throughout the medulla, contribute both inspiratory and expiratory potentials, and while they probably play in part on the respiratory motor centers, do not represent in themselves activity of the motor centers. Their wide distribution throughout the medulla would tend to obscure activity of the true motor centers.

CONCLUSIONS

1. Utilizing stimuli of moderate intensity, it is possible to perform an adequate physiological localization within the brainstem by the Horsley-Clarke method of stimulation, agreeing well with known morphology.

2. The functional subdivision of the respiratory center into inspiratory and expiratory portions previously proposed by Pitts, Magoun and Ranson (1939a) is affirmed to have a morphological basis.

REFERENCES

Beaton, L. E. and H. W. Magoun. This Journal **133:** P21, 1941.
Brookhart, J. M. This Journal **129:** 709, 1940.
Cordier, D. and C. Heymans. Ann. de physiol. **11:** 535, 1935.
Gesell, R. Ergebn. Physiol. **43:** 477, 1940.
Gesell R., J. Bricker and C. Magee. This Journal **117:** 423, 1936.
Hinsey, J. C. Res. Publ. Ass. Nerv. Ment. Dis. **20:** 657, 1940.
Magoun, H. W. and L. E. Beaton. This Journal **133:** P186, 1941.
Nicholson, H. C. This Journal **115:** 402, 1936.
Pitts, R. F., H. W. Magoun and S. W. Ranson. This Journal **126:** 673, 1939a.
This Journal **126:** 689, 1939b.

THE EFFECTS OF WATER MOCCASIN VENOM ON DOGS

ROBERT V. BROWN[5]

From the Department of Physiology and Pharmacology, Michigan State College[1]

Accepted for publication June 25, 1941

The action of the venom of the water moccasin, *Agkistrodon piscivorus* Lacépède, has received little physiological study. The principal work has been that of Mitchell and Reichert (1886), Flexner and Noguchi (1903), Noguchi (1909) and Essex (1932). The earlier workers found certain similarities between the actions of *Agkistrodon* and *Crotalus* venoms and noted the greater neurotoxic activity of the former. In his work on moccasin venom, Essex noted only similarities to the results from *Crotalus* venom as determined by him and his collaborators (Essex and Markowitz, 1930; Taube and Essex, 1937). Hence the neurotoxic action of the *Agkistrodon* venom is in question.

EXPERIMENTAL PROCEDURE. Dogs were anesthetized with sodium barbital,[2] 280 mgm. per kilogram of body weight, or sodium pentobarbital, 32 mgm. per kilogram, intravenously. Blood pressure was recorded by mercury manometer from the carotid artery, respiration by modified Marey tambour from the tracheal cannula. In some experiments, the phrenic nerves and one sciatic nerve were exposed. Animals were kept warm. The series of experiments is composed of thirty-one dogs.

The venom used was from the batch described by the author (1940). Venom was weighed out and diluted to 0.1 per cent with Ringer, Tyrode, or physiological saline; fresh solutions were made each time.[3] Dosage is expressed in milligrams of dry venom per kilogram of body weight. Injections were by cannula into the femoral vein and were washed in by burette. Duration of injections was variable.

In experiments in which the phrenic nerves were stimulated, the minimal break shock necessary to produce a visible respiratory effect was used. Similarly a minimal value for the tetanizing current was determined for the central end of a sciatic nerve; this value or a greater one was used in each case.

[1] A part of this work was done in the Departments of Physiology and Pharmacology, the University of Chicago.

[2] First four animals only.

[3] Venom-glycerol-Ringer stock solution (Essex, 1932) was used in the first two experiments.

RESULTS. On the basis of respiratory effects the animals may be divided into three groups: group I is composed of animals dying of immediate and absolute respiratory failure; group II consists of animals dying of respiratory failure but showing some respiratory activity, either terminal gasps or progressive depression, before complete failure; group III is composed of animals in which respiratory failure did not occur. These groups contain thirteen, forty-one, and forty-five per cent of the animals respectively.

Group I (fig. 1, A; fig. 2, no. 33). These animals showed fleeting stimulation, with immediate respiratory failure occurring within one minute of the beginning of the venom injection. There were neither terminal gasps nor response to sciatic stimulation: the failure was absolute. Minimal stimulations of the phrenic nerves produced twitches of the diaphragm.

There was an immediate severe fall in blood pressure within one minute of the beginning of the injection. A slow asphyxial rise then began in three cases; the fourth, which received the largest venom injection (1.25 mgm. per kgm.) showed no such rise. Sciatic stimulation usually lowered blood pressure.

The blood clotted well in two animals, poorly in one; the last was not autopsied. There was no hemorrhagic effect in any of them.

Group II (fig. 2, no. 24 and no. 10). These animals also showed respiratory failure; however, it either developed gradually or terminal gasps were present. Cheyne-Stokes breathing usually occurred in the first event. The effectiveness of sciatic stimulation varied directly with the degree of respiratory activity, being wholly ineffective once failure had occurred. Stimulations of the phrenic nerves produced twitches of the diaphragm after complete respiratory failure.

Blood pressure fell precipitately to forty-three per cent of the original at five minutes after beginning the venom injection. The extent of the asphyxial rise varied inversely with the survival time, being great in animals showing only terminal gasps and almost absent when the respiratory failure appeared very late. Sciatic stimulation usually lowered the blood pressure.

Only two animals were autopsied; one showed congestion; the blood clotted; the other showed occasional hemorrhages and a few clots.

Group III (fig. 1, B; fig. 3). These animals did not show respiratory failure; all received additional venom injections. The results discussed here are those of the first dose only.

The characteristic respiratory sequence after venom was: fleeting stimulation, depression (occasionally complete paralysis for a brief time, usually of the Cheyne-Stokes type), recovery, slight stimulation (approximately 30 per cent above the original), normal respiration. Again there was an inverse relationship between the effectiveness of sciatic stimulation and the degree of respiratory activity.

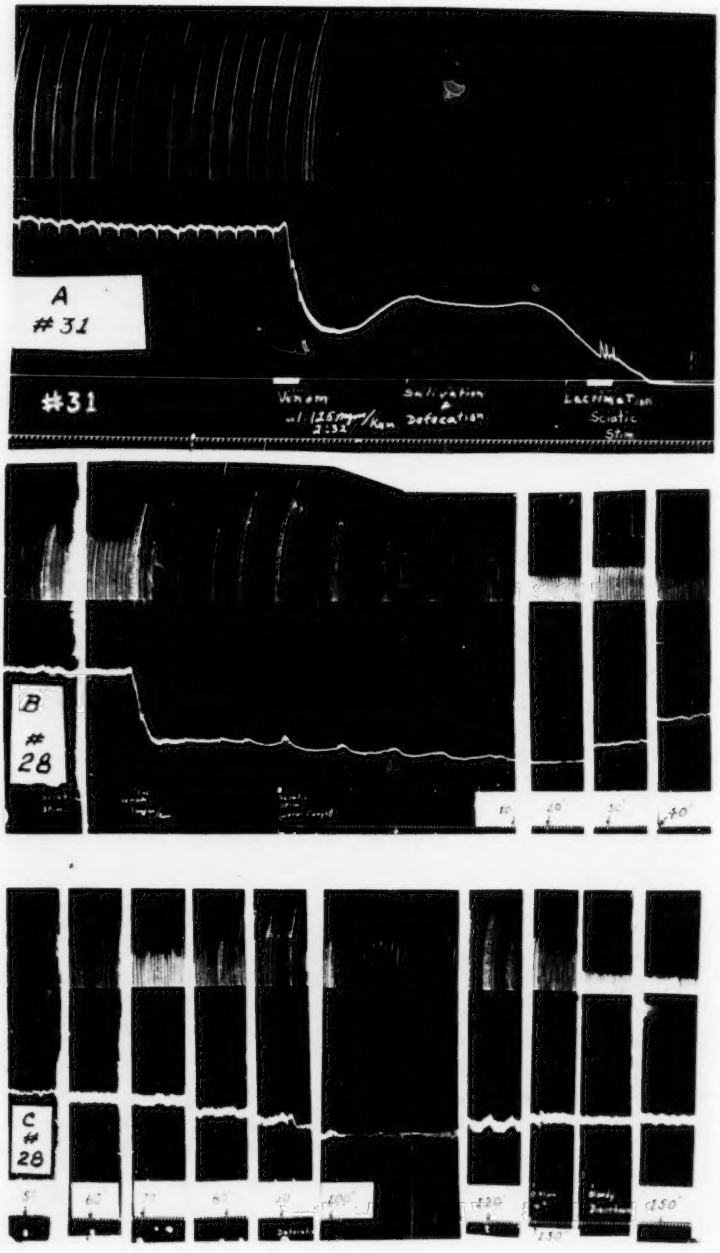


FIGURE 1

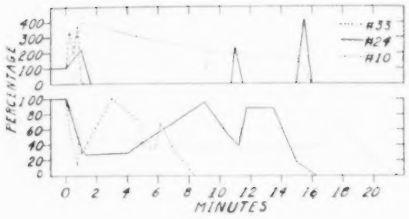


Fig. 2

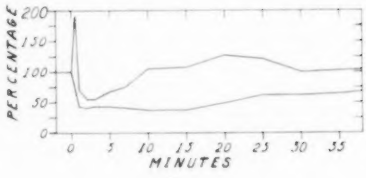


Fig. 3

Fig. 2. Ordinates are in percentages of the normal; abscissae are in minutes after beginning the injection of venom. The upper graph shows respiratory volume, i.e., the product of the rate and amplitude; the lower shows blood pressure.

No. 33 is from group I; note the early respiratory failure and the asphyxial rise in pressure. Venom: 1.125 mgm. per kilogram. (The fall in pressure at five minutes was caused by tetanizing stimulation of the central end of the sciatic nerve.)

Nos. 24 and 10 are from group II; the former shows terminal gasps, the latter early stimulation and gradual failure. Note the corresponding pressure changes. Venom: 1.0 and 0.5 mgm. per kilogram, respectively.

Fig. 3. Ordinates are in percentage of the normal; abscissae are in minutes after beginning of the venom injection. The upper line shows the respiratory effects, the lower the blood pressure effects in animals of group III. The respiration is the average for eleven animals; three showed pure stimulation and are excluded from the average. The blood pressure is the average for all the animals in the group.

TABLE 1

Miscellaneous data for the several groups

All numbers not otherwise designated are in percentage of the group. No average duration is shown for group III, since several injections were given each animal later; the effects of these are not shown here.

GROUP	PERCENTAGE OF TOTAL	SALIVATION	MICTURITION	DEFECATION	BRADYCARDIA	AVERAGE DURATION	PER CENT FALL IN BLOOD PRESSURE	AVERAGE DOSE
						minutes		*mgm./kgm.*
I	13	50	50	25	50	6.5	64	1.13
II	42	41	27	0	59	36.2	57	0.72
III	45	29	7	43	50	—	60	0.67

Circulatory changes correspond with those described by Essex (1932) for moccasin venom and, by inference, rattlesnake venom (Essex and Markowitz, 1930). The description by Taube and Essex (1937) of the

Fig. 1 A. Kymograph record of no. 31. Top line: respiration; second line: blood pressure; third line: zero pressure and record of events; fourth: time in five-second intervals. Anesthetic: sodium pentobarbital, 32 mgm. per kilogram. Venom dose: 1.125 mgm. per kilogram, so marked.

B and C. Kymograph record of no. 28. Lines are the same as in the tracing above. Minutes are marked on all sections except that for 140 minutes; the speed of the paper was changed at 100.25 minutes to show a group of respirations. Defecation occurred at four, ninety, and one hundred forty minutes; the last was mucous and bloody from intense gastro-intestinal hemorrhages.

gross pathology following the injection of rattlesnake venom is essentially applicable to the changes produced by moccasin venom in this group. Especially noteworthy were the absence of gross change in the lungs and the severe hemorrhagic destruction of the pancreas. Animals were only relatively refractory to additional venom injections.

Various data from the several groups are shown in the table.

Discussion. Taube and Essex (1937) and Essex and Markowitz (1930) showed that death from rattlesnake venom was caused by circulatory manifestations, i.e., "crotalin shock." Essex (1932) stated that *by the tests he employed*, "There is not a distinguishable difference in the physiologic action of the venom of the water moccasin and that of the rattlesnake." There is no discussion of respiratory effects either in his paper on moccasin venom or in the extensive series of Essex and his co-workers on rattlesnake venom.

Both the abrupt cessation of respiration in group I and the early or delayed failure in group II, accompanied by an asphyxial rise in blood pressure in both groups indicate acute respiratory paralysis as the cause of death. In view of the transitional nature of group II, the division between group II and III is somewhat arbitrary and is based on the presence of an asphyxial rise in group II which is absent in group III. On this basis 55 per cent of the animals died of respiratory paralysis. Even in group III, 79 per cent of the animals showed severe respiratory depression, with subsequent return to normal respiration. Ninety per cent of the entire series of animals showed severe depression or failure of respiration.

In all the animals in this series of experiments, a precipitate fall in blood pressure occurred; Essex and his co-workers ascribed this fall entirely to peripheral effects. In addition to these peripheral effects, there were central factors. The occurrence of marked asphyxial rises in pressure indicated that the vasomotor center, which had been depressed by the venom, was stimulated by the anoxemia consequent to the low blood pressure, with a concomitant pressure rise. When the asphyxia was delayed, this rise was less, probably because of at least three factors: 1, further venom depression of the vasomotor center; 2, permanent injury of the vasomotor center by the long-continued anoxemia consequent to the persistent low blood pressure; 3, appearance of hemorrhagic and other peripheral effects. When no asphyxia occurred, the survival time was long. Sciatic stimulation produced little vasomotor response, probably for the reasons enumerated above. Attempts to distinguish direct vasomotor depression from anoxemic injury have so far been unsuccessful.

Clotting of blood occurred in animals dying soon after venom injection; where death was delayed, as in group III, the blood remained fluid. Similarly hemorrhagic effects developed only in those animals which lived long enough for vascular destruction to occur (*ca.* 15 min.).

SUMMARY. In this series of dogs, 55 per cent of the animals died of respiratory failure brought about by direct action of the venom on the respiratory center.

Injections of venom caused an immediate severe fall in blood pressure in all cases. Animals dying of respiratory failure showed asphyxial rises in all cases but one.

After respiratory failure stimulation of the sciatic nerve produced no respiratory activity. Phrenic nerves were unaffected, as judged by the test employed; i.e., there is no evidence of a curare-like action.

Animals which had received one dose of venom were only relatively refractory to additional doses of venom.

Bradycardia was evident in 52 per cent of the subjects.

Salivation occurred in 38 per cent of the animals; defecation in 19 per cent; lacrimation was present in one case.

Clotting of the blood occurred only in animals dying within a few minutes after the injection; in others the blood remained fluid.

Hemorrhage into the tissues was absent in animals dying within a few minutes of the injection. The extent of hemorrhage varied directly with the duration of life.

CONCLUSION

Poisoning by water moccasin venom is partially due to the neurotoxic action which produces respiratory and vasomotor depression or failure; when these factors do not produce death, the persistent low blood pressure and the hemorrhagic vascular effects are responsible for the mortality. Between these extremes are cases where both sets of factors are active.

There is a definite relationship between the quantity of venom injected and the cause of death: the heavier doses yield the neurotoxic (central) effects and lighter doses the "shock" type (peripheral, or circulatory) effects.

REFERENCES

MITCHELL, S. W. AND E. T. REICHERT. Contrib. to Knowledge no. 27, Smithsonian Institution, 1886.

NOGUCHI, H. Carnegie Inst. Pub. no. 111, 1909.

FLEXNER, S. AND H. NOGUCHI. J. Exper. Path. Bact. **8**: 379, 1903. (Cited by H. NOGUCHI, loc. cit.)

ESSEX, H. E. This Journal **99**: 681, 1932.

TAUBE, H. N. AND H. E. ESSEX. Arch. Path. **24**: 43, 1937.

ESSEX, H. E. AND J. MARKOWITZ. This Journal **92**: 317, 239, 695, 698, 1930.

BROWN, R. V. This Journal **130**: 613, 1940.

THE RELATION OF EXTERNAL PANCREATIC SECRETION TO VARIATIONS IN BLOOD SUGAR[1]

V. BROWN SCOTT, U. J. COLLIGNON, H. J. BUGEL AND GRANT C. JOHNSON

From the Department of Physiology, Indiana University School of Medicine, Bloomington

Accepted for publication June 25, 1941

The effects of induced blood-sugar variations on external pancreatic secretion have been studied by numerous investigators with conflicting results. Babkin and Savitsch (1) showed that the intragastric administration of cane sugar and hydrochloric acid, to dogs with permanent pancreatic fistulae, elicited juice of a higher trypsinogen content than could be obtained by the acid alone. La Barre and Destrée (2), employing the cross-circulation method in chloralosed dogs, reported that moderate hypoglycemia of the recipient's encephalic centers diminished the volume and enzyme content of pancreatic juice elicited by intravenous secretin. This inhibitory effect was abolished by bilateral vagotomy. Gayet and Guillaumie (3) using a similar technique observed no increase in pancreatic secretion during hyperglycemia either before or after vagotomy. In anesthetized rabbits, Baxter (4) noted a decrease in the enzyme content of pancreatic juice obtained during insulin hypoglycemia. Vagotomy abolished this response but did not prevent the rise in enzymes evoked by hyperglycemia. Experiments conducted on men by Okada (5) and Frisk and Welin (6) showed the gastric and pancreatic secretion, collected by suction through intraduodenal and intragastric tubes, were augmented by insulin hypoglycemia. The former investigators showed that glucose inhibited this response while the latter obtained a greater rise in enzymes than in volume of pancreatic juice during insulin hypoglycemia.

From the evidence obtained by investigators (for a more complete review see Okada (5), Frisk and Welin (6)) it is evident that the relation of the external pancreatic secretion to blood-sugar variations has not been studied thoroughly in unanesthetized dogs with pancreatic fistulae. In order to obtain clean-cut evidence on this problem and to investigate some of the mechanisms involved, the following experiments were conducted.

METHODS. The majority of the pancreatic fistulae were prepared by the Inlow method. The details for the preparation of this fistula are given

[1] Portions of this paper were presented at the meetings of the American Physiological Society in March, 1940 and April, 1941.

elsewhere (7). In this fistula the transplanted major pancreatic duct permits easy cannulation at each experimental period. The animals were gastrostomized by the method of Carlson (8). Following postoperative recovery each animal was trained to lie on a padded table and pancreatic juice was conducted to an automatic drop counter and recorder by a glass cannula cemented into the transplanted duct with collodion. Intragastric pressure was recorded by a water manometer and condom balloon, the latter was introduced into the fundus of the stomach and inflated to a pressure of 5 cm. of water. The balloon was held in the fundus by a $\frac{1}{8}$ inch stiff-rubber tubing which passed through the balloon and extended beyond for several inches. This tube, with its perforated tip, served to drain fluid gastric content.

The other type of pancreatic fistula used in some of the experiments was prepared by introducing the special gastric and duodenal cannulae of Thomas (9). The duodenal cannula was placed in the intestine directly opposite the major pancreatic duct which permitted direct cannulation of the duct through the duodenal cannula (10). The special gastric cannula was placed in the fundus of the stomach which permitted the introduction of the balloon and drainage tube.

Observations were conducted for 3 to 6 hours on the trained animals which had fasted 18 to 24 hours. One unit of insulin per kilogram (Iletin or crystalline insulin) was injected subcutaneously or intravenously. Glucose (1 gram per kgm.) was injected intravenously, usually as a 20 per cent solution. Blood samples were drawn at appropriate intervals from the saphenous or cephalic vein and the blood sugar was estimated by the Shaffer-Hartmann method on cadmium sulphate filtrates.

Results. *Effects of subcutaneous insulin and intravenous glucose on the volume and proteolytic activity of pancreatic juice.* This group of experiments was conducted on 5 Inlow fistulae which were selected because they secreted large quantities of pancreatic juice and did not show "asecretory" periods (for a discussion of this problem see (11)). During experimental periods the pancreatic juice was collected at half-hour intervals and its proteolytic activity, after the addition of enterokinase, was estimated by formol titration. After a control period of 1 to 2 hours either insulin or glucose, or insulin followed in 50 to 60 minutes by glucose, was injected.

Figures 1, 2 and 3 are graphs of three results obtained. Figure 1 illustrates an experiment in which a reduction in the secretory rate and an augmentation of the proteolytic activity followed the administration of insulin. In the experiment graphed in figure 2 there was little difference in the volume of juice secreted following the insulin but again an increase in the proteolytic activity was observed. Figure 3 shows an increase in the volume and a decrease in the proteolytic activity of the juice collected after the administration of glucose. The data from these experiments are

summarized in table 1. In this table the average volume and the average proteolytic activity of the juice obtained after glucose or insulin administration is compared with the average values obtained during the corresponding control period. The result was recorded as an "increase," "decrease," or "no change."

These experiments demonstrated that no consistent, predictable results could be obtained with insulin or glucose under the above conditions. Babkin (12) has noted that in general the enzyme content of pancreatic

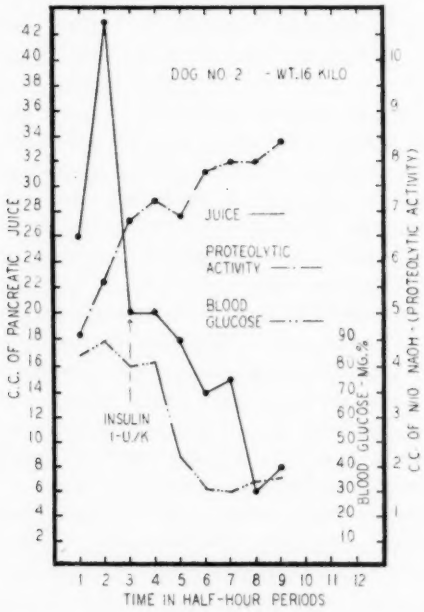


Fig. 1. Experiment showing a reduction in the volume and an increase in the proteolytic activity of pancreatic juice following subcutaneous insulin (1 unit per kgm.).

juice varies inversely with the volume of pancreatic juice. This observation was often confirmed by us in control experiments in which no insulin or glucose was injected. Since after insulin administration the proteolytic activity increased in 8 experiments it is possible that the method used above obscured small increases in this activity.

Effects of insulin and glucose on the volume of pancreatic secretion and on gastric motility. Numerous investigators (13, 14, 15, 16), have reported that insulin hypoglycemia augmented gastric motility; this hypermotility was usually inhibited by intravenous glucose. Since we (17) had observed

a temporal correlation between hunger contractions and pancreatic secretion it seemed possible that insulin might elicit an increase in the volume of pancreatic secretion concomitant with the onset of hypoglycemic gastric hypermotility.

Tables 2, 3 and 4 (group A) summarize the data obtained on 8 dogs employing the procedure outlined under Methods. These data show clearly that hypoglycemia at 50 to 60 minutes following subcutaneous or intravenous insulin was associated with an augmentation of both the gastric motility and the volume of pancreatic juice. This augmentation usually

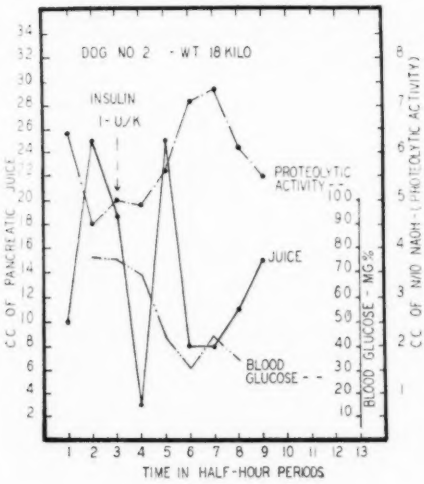


Fig. 2

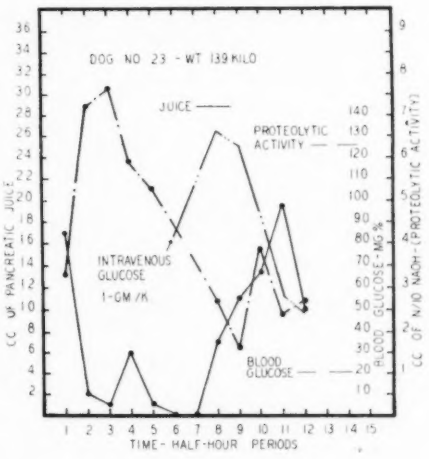


Fig. 3

Fig. 2. Results of an experiment in which no appreciable change in the volume was accompanied by an increase in the proteolytic activity of pancreatic juice after the administration of subcutaneous insulin (1 unit per kgm.).

Fig. 3. Experiment demonstrating an increase in the volume and a decrease in the proteolytic activity of pancreatic juice following intravenous glucose (1 gram per kgm.).

began when the blood sugar reached 40 or 50 mgm. per cent as is shown in figure 4. Usually, but not invariably, the gastric hypermotility preceded the pancreatic response by an interval of several minutes. Typically the hypermotility showed an incomplete tetany together with increased amplitude of gastric contractions. Glucose administered intravenously during the hypoglycemia produced an immediate reduction in the gastric and pancreatic activity (see fig. 4). In a few cases the pancreatic secretion was inhibited completely for 5 to 10 minutes. More often, however, the secretory rate was reduced and the gastric tone and contraction-amplitude was

diminished only to be followed in a short time by a return to a more rapid secretory rate and vigorous gastric contractions. This increase in pancreatic flow often occurred within 15 minutes after the injection of glucose and at a time when the blood sugar was still high. Injection of a hypertonic sodium chloride solution equal to the volume of the glucose solution failed to produce such inhibition and often produced an augmentation (see fig. 4). When a post-glucose hypoglycemia appeared, following the intravenous injection of glucose, the gastric and pancreatic responses were identical with those obtained during insulin hypoglycemia.

TABLE 1

Effect of subcutaneous insulin (1 unit per kgm.) and intravenous glucose (1 gram per kgm.) on volume and proteolytic activity of pancreatic juice

	SECRETION			ENZYME		
	Increase	Decrease	No change	Increase	Decrease	No change
Glucose, 16 experiments	4	10	2	4	3	9
Insulin, 16 experiments	8	5	3	8	3	5

TABLE 2

*Number of experiments showing the effect of 1 unit of insulin per kilogram subcutaneously at 50 to 60 minutes after injection on:**

GROUP	GASTRIC MOTILITY				PANCREATIC SECRETION			
	Augmentation			Inhibition	Augmentation			Inhibition
	Marked effect	Moderate effect	No effect		Marked effect	Moderate effect	No effect	
A	9	7	1	0	10	2	4	0
B	0	0	3	0	2	1	0	0

* Blood glucose 25 to 40 mgm. per cent.

Group A: Experiments on dogs before bilateral vagotomy.

Group B: Experiments on dogs after sectioning left vagus in the neck and right vagus in the thorax.

Regan (14) reported that an immediate transitory inhibition of gastric motility followed by the typical hypoglycemic augmentation appeared when intravenous insulin was administered. This reaction was observed in our experiments along with a concomitant inhibition of pancreatic secretion when either crystalline insulin or Iletin was used. While the initial pancreatic inhibition was sometimes complete, more often the secretory rate was merely diminished.

Effects of insulin and glucose on the volume of pancreatic secretion and on the gastric motility after bilateral vagotomy. In order to study the effects of

TABLE 3

Number of experiments showing the effect of intravenous injection of 1 unit of insulin per kilogram

GROUP	IMMEDIATE EFFECT ON:							
	Gastric Motility				Pancreatic Secretion			
	Inhibition			Augmentation	Inhibition			Augmentation
	Marked effect	Moderate effect	Little or no effect		Marked effect	Moderate effect	Little or no effect	
A	8	17	4	0	11	13	4	0
B	0	8	2	0	2	4	0	0
C	4	3	9	0	1	3	12	2
	EFFECT AT 50 TO 60 MINUTES FOLLOWING INJECTION ON:*							
	Gastric Motility				Pancreatic Secretion			
	Augmentation			Inhibition	Augmentation			Inhibition
	Marked effect	Moderate effect	Little or no effect		Marked effect	Moderate effect	Little or no effect	
A	17	5	2	0	17	7	5	0
B	0	0	9	0	5	4	0	0
C	0	0	17	0	0	0	17†	0

* Blood glucose 25 to 40 mgm. per cent.

Group A: Experiments on dogs before bilateral vagotomy.

Group B: Experiments on dogs after sectioning left vagus in the neck and right vagus in the thorax.

Group C: Experiments on dogs after *complete* vagotomy.

† Note: six of these showed a very *slight* augmentation.

TABLE 4

*Number of experiments showing the effect of 1 gram of glucose per kilogram intravenously on:**

GROUP	GASTRIC MOTILITY				PANCREATIC SECRETION			
	Inhibition			Augmentation	Inhibition			Augmentation
	Marked effect	Moderate effect	Little or no effect		Marked effect	Moderate effect	Little or no effect	
A	15	23	8	0	28	14	2	1
B	0	1	17	1	12	5	0	0
C	0	0	17	1	6	8	3	1

* Blood glucose 200 to 300 mgm. per cent.

Group A: Experiments on dogs before bilateral vagotomy.

Group B: Experiments on dogs after sectioning left vagus in the neck and right vagus in the thorax.

Group C: Experiments on dogs after *complete* vagotomy.

bilateral vagotomy on the gastric motility and pancreatic responses to insulin and glucose the following two-stage operations were performed and

the animals were studied, after recovery, under the conditions previously described. Two groups of animals were prepared: group B consisted of 4 incompletely vagotomized dogs (the right vagus was cut in the mid-thoracic region, the left was cut in the neck); group C was composed of 3 completely vagotomized dogs, two dogs having bilateral cervical vagotomies while the other had both vagi sectioned just above the diaphragm after a left cervical vagotomy.

The results of experiments conducted on the group B animals (see tables 3 and 4) showed that the immediate inhibitory effect of intravenous insulin on the gastric motility and pancreatic secretion usually persisted. The hypoglycemic effects, however, were markedly altered by the incomplete vagotomies since the rapid pancreatic secretory responses appeared but not the gastric hypermotility. The stomachs of these animals remained atonic

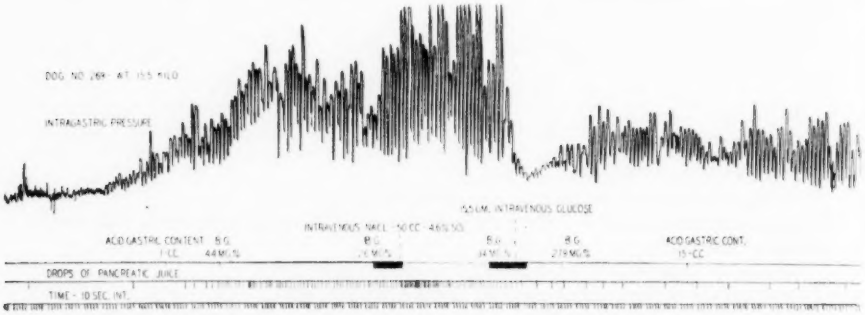


Fig. 4. Record of the effect of subcutaneous insulin (1 unit per kgm.), intravenous sodium chloride and glucose upon gastric motility and the volume of pancreatic juice (16 drops = 1 cc.).

during the rapid pancreatic secretion elicited by insulin hypoglycemia. These experiments prove that the pancreatic response to insulin hypoglycemia can be independent of the gastric motor activity.

In the completely vagotomized dogs (group C) the gastric motor response and the rapid pancreatic secretion of insulin hypoglycemia were both abolished (see group C, tables 3 and 4). In no experiment of this group was more than a very slight pancreatic augmentation elicited by insulin hypoglycemia. The initial inhibitory action of intravenous insulin, which was so frequently observed in the other experiments, was usually absent.

In both groups of animals (B and C) intravenous glucose produced no significant change in the atonic hypoglycemic stomach but almost always elicited a transient, marked or moderate reduction in the pancreatic secretory rate.

These experiments show that complete vagotomy abolishes the normal

motility and secretory response to insulin hypoglycemia but does not prevent an inhibitory action of intravenous glucose upon the pancreatic secretion. Complete vagotomy appears also to reduce the incidence of the inhibitory responses of the pancreas immediately following the intravenous injection of insulin.

The effects of excluding gastric juice from the duodenum upon the pancreatic secretory response to insulin hypoglycemia. Babkin (18) reported that insulin elicited a large flow of gastric juice which resulted from the hypoglycemic stimulation of the vagus centers because the gastric response was abolished by vagotomy. In view of this mechanism it seemed possible that the pancreatic response observed by us could have resulted from the entrance of gastric acid into the duodenum with the formation of secretin which called forth a rapid pancreatic secretion. In order to determine whether this mechanism played a part in the hypoglycemic pancreatic response the following experiments were performed. Because vagotomy can alter the gastric and pancreatic responses to insulin it was necessary to exclude gastric juice from the duodenum without extensive damage to the nerve supply of the stomach or pancreas. To avoid such damage the 3 dogs with gastric and pancreatic fistulae were subjected to sub-muscular "pyloric separations". In this operation the thick muscular layers of the stomach were incised longitudinally and completely separated from the underlying submucosa at a level just rostral to the pyloric sphincter. The gastric mucosa and submucosa were divided and the cut ends were so closed as to completely separate the lumen of the stomach from the duodenum. The longitudinal incision was carefully sutured to avoid extensive destruction of the nerves which are present in this region.

In 6 experiments conducted on these animals insulin produced a typical gastric hypermotility during hypoglycemia but failed to augment the rate of pancreatic secretion significantly. In no instance was the rapid pancreatic secretion observed which was the characteristic response of these animals prior to pyloric separation. These experiments were conducted on the animals after healing of the abdominal wounds had occurred. In a number of semi-acute experiments insulin failed to initiate either gastric hypermotility or pancreatic augmentation. These experiments demonstrate that closure of the stomach at the pylorus prevents the rapid secretory response of the pancreas normally seen during insulin hypoglycemia.

The relation of "spontaneous" blood-sugar variations to gastric motility and pancreatic secretion. E. B. Boldyreff (19) reported that during the "work" periods of the stomach and pancreas the blood sugar fell 20 to 30 mgm. per cent and returned to higher levels during the "rest" periods of these organs. Mulinos (16) (on dogs) and W. W. Scott (20) (on men) failed to establish any causal relation between the blood-sugar level and gastric hunger motility. In order to extend these observations to include fasting

pancreatic secretion 30 experiments on 7 dogs were performed; in 11 of these experiments the pancreatic secretion was led to the exterior and recorded; in the remainder of the experiments the pancreatic juice entered the duodenum naturally and only the motility of the stomach was recorded. Table 5 shows a typical experiment in which no significant changes in blood sugar occurred during marked gastric and pancreatic activity. It will be noted that the pancreatic flow ceased during the intermotility period and that the blood sugar was higher during the subsequent period of hunger contractions than during the period of quiescence of the stomach and pancreas. The experiments in which the pancreatic juice was not diverted from the intestine also failed to show the positive correlation between the blood sugar and the gastric motility claimed by Boldyreff.

TABLE 5

Blood sugar, gastric motility and pancreatic secretion

TIME	BLOOD SUGAR	MAXIMUM INTRAGASTRIC PRESSURE	DROPS OF PANCREATIC JUICE PER MINUTE
	milligrams per cent	*centimeters of water*	
8:15 a.m.	66	27	7
9:01 a.m.	65	29	12
9:33 a.m.	76	33	10
10:13 a.m.	76	6*	0
10:48 a.m.	80	15	2
11:10 a.m.	75	23	11

* Stomach showed no hunger motility during this period.

Discussion. It is evident from the foregoing observations that insulin produces a rapid flow of pancreatic juice, together with gastric hypermotility, when the blood sugar is sufficiently reduced. The consistent appearance of the gastric motility response to hypoglycemia confirms the observations of previous investigators (13, 14, 15, 16). In our initial experiments the use of dogs which were "hypersecreting" (Babkin, 12) probably accounts for our original failure to demonstrate the stimulating effects of insulin hypoglycemia on the external pancreatic secretion.

The effects of vagotomy on the volume of pancreatic juice secreted appear to depend upon the completeness of the vagotomy. When both vagi are sectioned in the neck the motility of the stomach and the increased pancreatic secretion seen in insulin hypoglycemia are lost. On the other hand, incomplete vagotomy does not abolish the pancreatic response but only the typical gastric hypermotility of hypoglycemia. The occasional very slight increases in the pancreatic secretion, seen during hypoglycemia in the completely vagotomized dog, were always so small as to be questionable increases. Contrary to Boldyreff (21) we found that insulin

elicited a hypoglycemia after complete vagotomy. Our results confirm, in general, the observations of Okada (5) made on human subjects. These results were less precise than ours because the aspirated pancreatic juice was contaminated with bile and succus entericus.

The immediate inhibitory action of intravenous insulin on gastric motility and pancreatic secretion is most marked and most frequent in the animals before vagotomy. Even incomplete vagotomy often reduces the magnitude of this inhibition. In a few experiments on completely vagotomized dogs the pancreatic flow accelerated immediately after the intravenous injection of insulin. It is possible that intravenous insulin stimulates the centers which control the vagus constrictor fibers to the pancreatic ducts. Special experiments designed to investigate this problem will be needed to settle this question.

The transient inhibitory action of intravenous glucose on the pancreatic secretion persists after complete vagotomy. It seems probable that this inhibitory effect of glucose is exerted directly on the pancreas because this effect persisted in several splanchnicotomized dogs. In 6 experiments intravenous glucose inhibited the spontaneous pancreatic secretion, the inhibition lasting for as long as 20 minutes but usually for a shorter period. The effect resembled the transient inhibitory effect of glucose seen during hypoglycemia.

In considering the way in which the pancreas is excited to secrete rapidly during hypoglycemia it is necessary to consider the secretin mechanism which could play an important rôle in this response. Complete vagotomy abolishes the typical hypoglycemic pancreatic response and also abolishes the typical gastric secretion under these conditions. These results could be explained on the removal of the secretin mechanism. In animals with pyloric separations no gastric juice can enter the duodenum; these animals react to insulin hypoglycemia exactly like the completely vagotomized dogs. This is strong evidence for the dependence of the pancreatic response upon the gastric secretory response. The simplest explanation of these results is that in both conditions the normal secretin mechanism has been eliminated. Frisk and Welin (6) failed to observe marked pancreatic responses in men during insulin hypoglycemia, when the gastric content was continuously aspirated by the gastric tube. Okada (5), however, has observed hypoglycemic pancreatic augmentation in cases of gastric carcinoma which secreted alkaline gastric juice. These results are difficult to reconcile with our observations. Additional experiments, designed to detect the liberation of secretin under the above conditions, are needed definitely to settle this problem.

The authors are indebted to Dr. F. B. Peck, Lilly Research Laboratories, and to Dr. H. Jensen, Squibb Institute for Medical Research, for the Iletin and crystalline insulin used in these experiments.

CONCLUSIONS

1. Insulin hypoglycemia increases the volume of pancreatic juice secreted by unanesthetized dogs having permanent pancreatic fistulae.

2. Intravenous glucose temporarily inhibits the pancreatic secretion which appears spontaneously or in response to insulin.

3. Complete bilateral vagotomy abolishes the rapid flow of pancreatic juice and the gastric hypermotility of insulin hypoglycemia; incomplete vagotomy abolishes the gastric but not the pancreatic response.

4. Intravenous insulin exerts a transient inhibitory effect on the fasting gastric motility and pancreatic secretion; the incidence of this effect is reduced by complete vagotomy.

5. Exclusion of the gastric juice from the duodenum by pyloric separation abolishes the rapid pancreatic secretion of insulin hypoglycemia.

6. Spontaneous variations in the volume of the fasting pancreatic secretion and gastric motility are unrelated to the fluctuations in blood sugar.

REFERENCES

(1) Babkin, B. P. and W. W. Savitsch. J. russe de physiol. **3:** 143, 1921.
(2) La Barre, J. and P. Destrée. Compt. rend. Soc. de biol. **98:** 1237, 1240, 1928.
(3) Gayet, R. and M. Guillaumie. Compt. rend. Soc. de biol. **103:** 1220, 1930.
(4) Baxter, S. G. Quart. J. Exper. Physiol. **21:** 355, 1932.
(5) Okada, S. Arch. Int. Med. **43:** 446, 1929; **45:** 783, 1930.
(6) Frisk, A. R. and G. Welin. Acta Med. Scand. **91:** 170, 1937.
(7) Scott, V. B. J. Lab. and Clin. Med. **25:** 1215, 1940.
(8) Carlson, A. J. The control of hunger in health and disease. Chicago, 1916.
(9) Thomas, J. E. Proc. Soc. Exper. Biol. and Med. **46:** 260, 1941.
(10) Scott, V. B. Unpublished method.
(11) Scott, V. B., J. S. Graham and D. H. McCartney. Am. J. Diges. Dis. **7:** 533, 1940.
(12) Babkin, B. P. Die Äussere Sekretion der Verdauungsdrüsen. Berlin, 1928.
(13) Bulatao, E. and A. J. Carlson. This Journal **69:** 107, 1924.
(14) Regan, J. F. This Journal **104:** 90, 1933.
(15) Quigley, J. P., V. Johnson and E. I. Solomon. This Journal **90:** 89, 1929.
(16) Mulinos, M. G. This Journal **104:** 371, 1933.
(17) Scott, V. B., C. C. Scott and H. J. Bugel. This Journal **131:** 60, 1940.
(18) Babkin, B. P. Am. J. Digest. Dis. **5:** 753, 1939.
(19) Boldyreff, E. B. Pflüger's Arch. **218:** 553, 1928.
(20) Scott, W. W., C. C. Scott and A. B. Luckhardt. This Journal **123:** 243, 1938.
(21) Boldyreff, E. B. and J. F. Stewart. J. Pharmacol. and Exper. Therap. **46:** 407, 1932.

THE PRODUCTION OF EXPERIMENTAL POLYCYTHEMIA IN DOGS, RABBITS AND MAN BY THE DAILY ADMINISTRATION OF EPHEDRINE; AND BY AMPHETAMINE IN DOGS

JOHN EMERSON DAVIS

From the Department of Physiological Chemistry and Pharmacology, University of Vermont College of Medicine, Burlington

Accepted for publication June 27, 1941

We have shown previously (1) that choline and other vasodilator drugs are effective in depressing the experimental polycythemias produced in dogs by cobalt administration or by daily exposure to low atmospheric pressure. To explain this action of the above drugs, we assume that they improve the blood flow to bone marrow, thus diminishing the local anoxia and removing the stimulus to polycythemia.

If this explanation is correct, it would seem that drugs which might constrict marrow arterioles should have the opposite effect; i.e., should increase the rate of red blood cell formation.

The present investigation was made, therefore, to see whether a polycythemia could be produced by certain drugs which have the general action of producing vasoconstriction. Ephedrine and amphetamine (benzedrine) were chosen for this trial because of their prolonged action and resistance to destruction by the body.

PROCEDURE. The subjects used for these experiments consisted of 6 dogs, 7 rabbits and one human. The dogs and rabbits were fed a constant adequate diet and were allowed water ad libitum.

Control observations on the blood were made over a period of at least two weeks before the drug administrations were commenced. These observations included erythrocyte counts, hemoglobin determinations (Sahli), total leukocyte counts, and estimation of the percentage of reticulocytes. The latter observation was made using glass slides on which a drop of blood had been mixed and smeared with a drop of aqueous cresyl blue solution, dried, and then stained with Wright's stain.

Blood samples were drawn from the saphenous vein in the dogs, and only at times when the animals were in an unexcited and basal state. In the rabbits blood was drawn directly into diluting pipettes from the site of puncture of the marginal ear vein. During the experimental periods, blood was sampled only after the elapse of at least 17 hours from the time at which the daily dose of drug had been given.

Ephedrine sulfate was administered to four dogs by stomach tube in daily doses ranging from 2.5 to 5.0 mgm. per kilogram. The latter dose level has been shown by Ogden and Teather (2) to raise the blood pressure in dogs following oral or subcutaneous administration.

In similar experiments on rabbits, 2 normal and 2 splenectomized rabbits were given 45 mgm. of ephedrine sulfate daily by subcutaneous injection.

Amphetamine (Benzedrine) sulfate was given orally to one splenectomized and 3 normal dogs in a daily dose of 10 mgm. The same daily dose was administered by stomach tube to 4 rabbits, 2 of which were splenectomized.

Three rabbits with cobalt polycythemia were given daily injections of 45 mgm. of ephedrine sulfate in addition to daily injections of 10 mgm. of

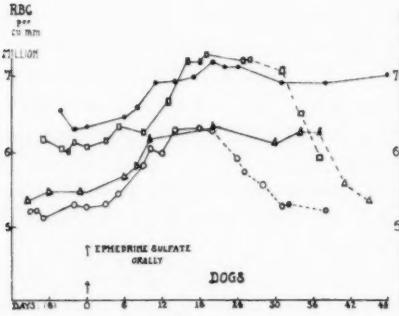


Fig. 1

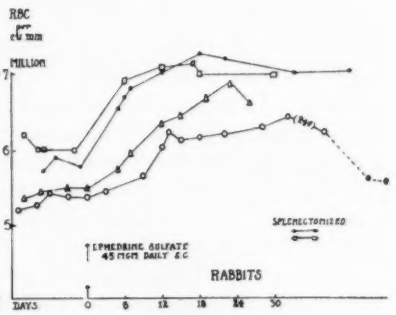


Fig. 2

Fig. 1. The production of polycythemia in dogs by the daily administration of 2.5 to 5.0 mgm. of ephedrine sulfate per kilogram by stomach tube. Dashes indicate cessation of ephedrine feeding.

Fig. 2. The effect of daily subcutaneous injection of 45 mgm. of ephedrine sulfate into 2 normal and 2 splenectomized rabbits, upon their red blood cell counts.

cobalt (40 mgm. cobalt chloride) which was the dose used to induce this type of polycythemia.

One human subject ingested ephedrine sulfate in a daily dose of 50 mgm. for 20 days.

Results. The daily oral administration of ephedrine to 4 normal dogs caused an increase of about one million in their basal erythrocyte numbers (fig. 1). The development of polycythemia was gradual. No significant increase of red cells occurred during the first 6 days. Within 10 to 15 days, however, the full increases in red cell numbers were apparent. They persisted throughout the periods of ephedrine administration and dropped back to normal within 7 to 10 days after the discontinuation of ephedrine. The cessation of the drug feeding in each dog is indicated by the beginning of the dashes in figure 1. Hemoglobin percentages (not shown) ran parallel

with the red cell counts, throughout. The reticulocyte percentages were approximately doubled during the polycythemia.

Total leukocyte counts remained approximately constant for each dog and did not vary by more than 10 per cent throughout the experiments. The counts are not shown here, but the normal values ranged from 13000 to 15000 per cu. mm. of blood.

Figure 2 shows the effect of daily injections of 45 mgm. of ephedrine sulfate upon the erythrocyte numbers of 2 normal and 2 splenectomized rabbits. Within one to two weeks the red cell counts of all 4 rabbits had increased by about one million. Reticulocyte percentages (not shown) were also increased from a normal value of 1.5 (average) to 4.0 during polycythemia.

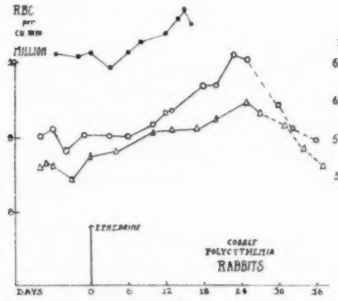


Fig. 3

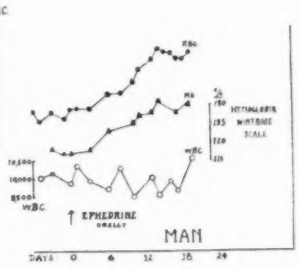


Fig. 4

Fig. 3. The additive effect of daily injections of ephedrine (45 mgm., subcutaneously) upon the blood of rabbits with cobalt-induced polycythemia.

Fig. 4. The effect of daily ingestion of 50 mgm. of ephedrine sulfate upon the blood of one human subject.

Ephedrine sulfate (45 mgm. daily) was injected into three rabbits which already had cobalt polycythemia (fig. 3). This produced further increases in the red cell counts, which amounted to about one half to one million cells per cubic millimeter in the different animals. The cobalt injected rabbits received 10 mgm. of cobalt daily in the form of the chloride; and at the time at which ephedrine injections were started, each rabbit had a polycythemia of about 50 per cent, which had required about two and a half months to develop.

One human subject (the author) took ephedrine sulfate by mouth in a daily dose of 50 mgm., with results on the blood which are shown in figure 4. An increase of about 0.8 million in the erythrocyte number was produced in two weeks. This was accompanied by an increase in hemoglobin of about 11 per cent. Total leukocyte counts, however, did not go up but remained fairly constant around a value of ten thousand. In this experi-

ment it was observed that the first few daily doses of ephedrine raised systolic blood pressure 15 to 20 mm., diastolic pressure about 5 mm., and slowed the pulse rate by about 10 beats per minute. These changes commenced about 40 minutes after ingestion of the drug and persisted for at least 2 hours. Subsequent daily doses of ephedrine did not appear to alter the systolic pressure very markedly. No unpleasant symptoms were experienced as a result of taking the drug. An E.C.G. taken one hour and fifty minutes after the 20th daily dose of ephedrine appeared to be quite normal.

Amphetamine sulfate was given orally to one splenectomized and 3 normal dogs in a daily dose of 10 mgm. As can be seen in figure 5, all four dogs developed polycythemia within 12 days. Erythrocyte counts increased by about one million while hemoglobin percentages (not shown)

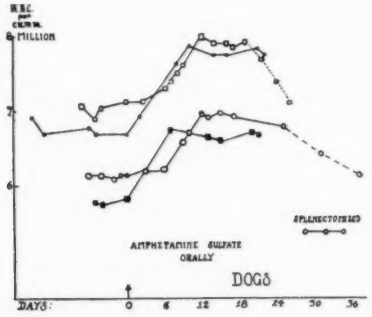


Fig. 5. The development of experimental polycythemia in dogs by the daily oral administration of 10 mgm. of amphetamine sulfate. Dashes indicate cessation of drug feeding.

increased, but not so greatly. Recovery from polycythemia is not shown for 3 dogs in figure 5, since it was desired to do other experiments on these animals. In the one animal in which recovery is illustrated (fig. 5), about 12 days were required for this to occur.

Amphetamine sulfate (10 mgm. daily) was also fed to 4 rabbits by stomach tube. Two of these rabbits apparently developed a polycythemia, but the other two did not, although they received the drug for about eighteen days. Since the results were inconclusive, they are not shown in this paper.

Discussion. The slow onset of polycythemia in dogs, rabbits and man following the commencement of daily ephedrine administration as well as its slow regression upon cessation of the drug indicates a probable true increase in erythropoiesis. This interpretation is also supported by the reticulocytosis observed in dogs and rabbits during these experiments.

The fact that splenectomized animals responded in the same way as normals to ephedrine or amphetamine also supports our belief that these drugs stimulate red blood cell production.

The remote possibility that ephedrine might cause a slow chronic change in state of blood reservoirs and thus increase the erythrocyte number, is largely precluded by the appearance of polycythemia in our splenectomized animals (figs. 2 and 5). Furthermore, the induction of polycythemia in man by ephedrine (fig. 4) does not seem explainable on this basis when viewed in the light of recent work by Ebert and Stead (3). These authors present evidence tending to show that the erythrocytosis observed in normal men as an acute response to exercise or epinephrine injection is not due to contraction of blood reservoirs. Their evidence even casts doubt upon the existence of blood (storage) reservoirs in the human organism.

Dehydration of the blood probably plays no part in the polycythemias reported in this paper. In the first place, fluid loss by the blood might be expected to occur after the first daily dose of drug, if at all; but no significant increase of basal erythrocyte numbers occurred in any of our animals during the first week (figs. 1–5). Secondly, the relative constancy of normal leukocyte counts in man (fig. 4) and dogs (not shown) during ephedrine polycythemia, seems to argue against a possible blood concentration.

The additive effect produced by ephedrine upon rabbits with cobalt-induced polycythemia (fig. 3) would appear to show that this drug stimulates erythropoiesis by a different mechanism than the metal. A theory on the mechanism of cobalt polycythemia has previously been advanced by the author (4) who found that it differed from the polycythemia induced by exposure to low atmospheric pressure, in certain respects. The cobalt polycythemias in rabbits (fig. 3) which represent increases of approximately 50 per cent over the normal erythrocyte numbers, are incidentally greater in extent than any which we have found reported for this species in the literature.

As to the mechanism by which ephedrine stimulates bone marrow, the most plausible explanation seems to reside in a reduction of blood flow to this tissue through vasoconstriction, with a consequent diminution of its oxygen supply. We have previously shown (1) that choline and its derivatives may have the opposite effect, i.e., they depress excess erythropoiesis in polycythemic dogs, probably by improving the blood supply to bone marrow, since their action is blocked by atropine.

Local hypoxia of bone marrow as the stimulus to polycythemia by ephedrine seems plausible from the standpoint of the *time required* to induce the full rise in the erythrocyte number, which agrees generally with the time required to induce polycythemia in dogs by exposure to low atmospheric pressure (5). It may be assumed, perhaps, that the resultant

immediate stimulus to polycythemia is the same in either case (i.e.) hypoxia of marrow.

According to Warren (6) bone marrow metabolism is not under nervous control in rabbits exposed to low atmospheric pressure. We may assume that ephedrine does not act through the nervous system, but rather directly on marrow arterioles.

The mechanism of the production of polycythemia by benzedrine (amphetamine) in dogs is probably the same as that of ephedrine, since the responses of the red cell counts are the same for each drug.

SUMMARY AND CONCLUSIONS

The daily oral administration of ephedrine sulfate to 4 normal dogs, in doses ranging from 2.5 to 5 mgm. per kilogram, caused significant increases in the basal erythrocyte numbers of all of the animals within 10 to 15 days. The red cell counts and hemoglobin percentages remained elevated throughout the period of ephedrine feeding (3 to 7 weeks, or more) and returned to their normal values within 7 to 10 days after cessation of drug administration. Total leukocyte counts remained fairly constant.

Two normal and two splenectomized rabbits which received 45 mgm. of ephedrine sulfate daily by subcutaneous injection, developed polycythemia with reticulocytosis, within two weeks.

The daily injection of ephedrine into 3 rabbits which had a marked cobalt-induced polycythemia, produced further appreciable increases in their erythrocyte numbers.

A significant polycythemia was produced in one human subject in 2 weeks by the daily ingestion of 50 mgm. of ephedrine sulfate. The total leukocyte count did not increase, or change significantly.

The oral administration of 10 mgm. of amphetamine sulfate daily to one splenectomized and 3 normal dogs caused significant increases in their basal red cell counts and hemoglobin percentages within 12 days.

These results can be explained by assuming that ephedrine and amphetamine reduce the blood flow to bone marrow, thus diminishing its oxygen supply, and thereby stimulating erythropoiesis.

Acknowledgment. The author wishes to express his appreciation to Prof. H. B. Pierce for valuable suggestions made in connection with this work, and to Messrs. Dean Wheeler and John Prybylo for technical assistance.

REFERENCES

(1) Davis. J. Pharmacol. and Exper. Therap. **70:** 408, 1940.
(2) Ogden and Teather. J. Pharmacol. and Exper. Therap. **54:** 320, 1935.
(3) Ebert and Stead. Am. J. Med. Sc. **201:** 655, 1941.
(4) Davis. This Journal **129:** 140, 1940.
(5) Davis. This Journal **127:** 322, 1939.
(6) Warren. Personal communication.

CATION DISTRIBUTION IN THE MUSCLES OF ADRENALECTOMIZED RATS[1]

MARY V. BUELL AND ELEANOR TURNER

From the Chemical Division, Department of Medicine, The Johns Hopkins Hospital and The Johns Hopkins University, Baltimore, Md.

Accepted for publication June 28, 1941

The changes in both the serum electrolyte pattern and also in the electrolyte balance associated with adrenal insufficiency, either untreated or treated in a variety of ways, have been the subject of intensive study by many investigators. Also certain phases of carbohydrate metabolism have been shown to be under the direct hormonal regulation of the adrenal cortex (1, 2). Appreciating the definite but poorly understood correlation between normal carbohydrate metabolism and changes in the concentration of certain serum electrolytes the authors felt that much might be learned concerning the fundamental nature of adrenal insufficiency and perhaps also of normal carbohydrate metabolism by the analysis of the tissues themselves (muscle and liver) for electrolytes as well as for the intermediary products in the metabolism of carbohydrates. To this end they collected the skeletal muscles and livers of the same rats whose carbohydrate metabolism was reported upon in 1936 and analyzed these tissues for the four common cations of known physiological importance, namely, sodium, potassium, calcium and magnesium. Such significant changes in the cation contents of the muscles (but not in the livers) were found that the authors were unwilling to report the results at that time (1937) without further amplification.

In the meantime, tissue analyses for electrolytes have been reported by other workers. Harrison and Darrow (3) found, as had we, that there were greatly increased amounts of potassium and somewhat decreased amounts of sodium in the skeletal muscles of rats in adrenal insufficiency.

EXPERIMENTAL. In the experiments to be reported in detail the procedure was as follows: Male albino rats of the Sprague Dawley strain were kept for two weeks or more under laboratory conditions on a diet consisting of Purina Dog Chow[2], on which they grew rapidly. The animals were

[1] This study was aided by a grant to Dr. George W. Thorn from the Committee on Research in Endocrinology, National Research Council.

[2] The cation content of this food was reported by Dr. H. J. Smith to be: Mg 0.09 per cent, K 0.56 per cent, Na 0.67 per cent and Ca 1.5 per cent.

young, but were permitted to grow to a weight of 250 to 300 grams in order that there might be enough muscle available to permit the analysis for the four cations in the muscles from the same animal. Bilateral adrenalectomy was performed under nembutal anesthesia by the lumbar route[3]. In one group of animals a pellet weighing 80 mgm. of pure synthetic desoxycorticosterone acetate[4] was inserted subcutaneously at the time of operation. During the period of recovery from the operation all of the rats were given 0.9 per cent sodium chloride solution to drink instead of water. Tap water was then substituted for saline in those animals in which adrenal insufficiency was allowed to develop. After a period of seven days without specific therapy the animals were sacrificed without a period of fasting regardless of the degree of insufficiency which had developed. In no instance was the animal moribund. Each animal was subjected to the same routine. Sodium nembutal was injected intraperitoneally. When anesthesia was complete, the heart was exposed and blood was withdrawn under oil into a syringe[5]. The animal was then skinned and the skeletal muscle was dissected from the bones, care being taken that corresponding muscles were used in all cases. The finely cut-up muscle was introduced into a tared flask, ashing and analysis being carried out as described in a previous paper (4). In these experiments, calcium was precipitated as oxalate at pH 4.2 in a centrifuge tube. The supernatant fluid and washings were collected in a 100 ml. volumetric flask, made up to volume, and appropriate aliquots taken for the determination of magnesium by the estimation of the phosphorus in precipitated magnesium ammonium phosphate. Duplicate determinations were run of potassium and magnesium, but not of sodium and calcium.

Since potassium was found to be the cation which showed the largest quantitative variations in the muscles of the different groups of animals the results have been reported in a series of charts designed to illustrate the simultaneous variations between potassium and each of the other cations in individual animals. A summary of the detailed data, presented graphically in the charts, has been recorded in table 1 as average figures for the indicated number of animals. The points illustrated by the charts and table will be discussed in detail.

Discussion. *Variations in the normal values of muscle potassium.* One unexpected feature of these results was the great variability of the values for muscle potassium in all of the groups studied including the group of normal animals[6]. If the values plotted on the abscissa in figure 1 are

[3] The authors are indebted to Dr. Roger Lewis and to Dr. George Koepf for assistance with the operations and care of the animals.

[4] The synthetic desoxycorticosterone acetate used in this study was kindly supplied by Dr. Ernst Oppenheimer of the Ciba Pharmaceutical Products, Inc.

[5] The blood from animals similarly treated was pooled.

[6] This point would have been missed completely had pooled specimens been analyzed.

disregarded for the moment, the values for potassium may be read on the ordinate. Because of the large range in potassium values a suitable scale was necessarily chosen to permit the presentation of all of the points obtained. In figure 2 the same data have been plotted with the omission of the points representing the animals in adrenal insufficiency. Here the scale representing potassium values permits a ready demonstration of the range of variations found among the normal animals (open triangle).

According to the Conway-Boyle theory (5), the muscle cell is normally freely permeable to the potassium ion. Also the concentration of cellular

TABLE 1

Summary of analysis of skeletal muscles of rats

NUMBER OF ANIMALS	CONDITION OF ANIMALS	SPECIFIC THERAPY	MOIST SKELETAL MUSCLE (GRAMS PER CENT)		DRY, FAT-FREE MUSCLE (MEQ. PER 100 GRAMS)					SERUM				
			Moisture	Lipid	Na	K	Ca	Mg	Total base	Na per liter	K per liter	Ca per liter	Cl per liter	N.P.N. per 100 ml.
										meq.	*meq.*	*meq.*	*meq.*	*mgm.*
11	Normal	None	75.0	1.7	10.0	32.0	1.4	8.3	51.7	144.2	6.6	5.5	98.4	34
4	Normal	Desoxycorticosterone acetate pellets	74.3	2.3	14.1	30.2	1.1	7.8	53.2	142.7	6.2	5.3	88.4	34
11	Adrenalectomized compensated	Sodium chloride	75.2	1.9	9.5	36.1	1.2	7.1	53.9	143.4	7.0	5.2	107.3	48
8	Adrenalectomized compensated	Desoxycorticosterone acetate pellets	75.8	1.3	15.6	29.9	1.4	6.8	53.7	141.4	7.8	4.3	93.4	36
11	Adrenalectomized. In insufficiency	None	76.0	1.1	8.9	118.1	1.4	5.9	134.3	133.7	8.5	5.8	94.0	46

potassium would be expected to vary in different phases of carbohydrate metabolism since "the impermeable non-colloidal anions are mostly phosphorylated compounds important for the carbohydrate cycle. If such compounds decrease in concentration during rapid carbohydrate oxidation, potassium should leave the cell, and when reformed the reverse should occur." It seems reasonable to expect, therefore, that there should be large variations in the muscle potassium of normal unfasted animals in different phases of carbohydrate metabolism. The data suggest that in normal animals the muscle potassium falls into two categories; 1, a constant minimum quantity (about 26 meq. per 100 grams of dry fat-free

muscle), and 2, a variable quantity (which in these experiments was represented by increments amounting sometimes to as much as 20 meq.). The former may represent the potassium which is held within the cells in the basal condition (i.e., "basal potassium"), the latter may represent that which enters the cells to meet the changing temporary demands of carbohydrate metabolism (i.e., "metabolic potassium").

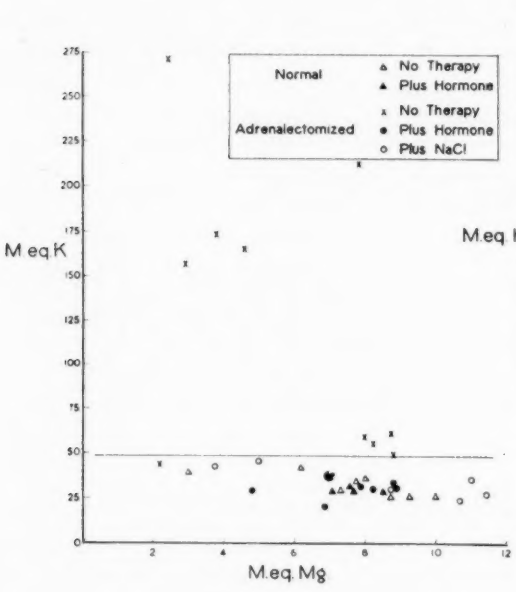


Fig. 1

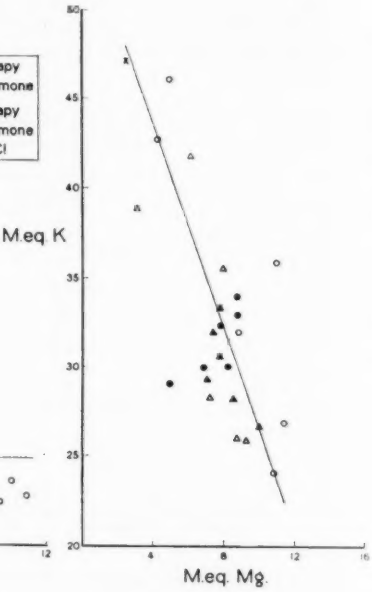


Fig. 2

Fig. 1. Gross relationship between potassium and magnesium in the whole skeletal muscles of all of the animals studied, values being reported as milliequivalents of cation per 100 grams of dry fat-free muscle. The hormone used was crystalline synthetic desoxycorticosterone acetate.

Fig. 2. Detailed relationship between potassium and magnesium in the whole skeletal muscles of all of the animals which were "compensated," values being reported as milliequivalents of cation per 100 grams of dry fat-free muscle. The hormone used was crystalline synthetic desoxycorticosterone acetate.

Variations in the muscle potassium of animals in adrenal insufficiency. Also, among the adrenalectomized animals which were given no specific therapy (fig. 1) the increases in muscle potassium were much greater in certain instances than were those found in our previous experiments with the four-hour fasted rats *in extremis*, or those reported by Harrison and Darrow. The animals with the highest muscle potassium values were not always those which were suffering from the most severe degree of insuffi-

ciency as judged by the weight loss. The suggestion is offered that the high muscle potassium might have been the result of the fact that these animals were still eating and therefore were still attempting to metabolize exogenous carbohydrate with the concomitant accumulation in the muscle cells of large quantities of impermeable anions which held potassium imprisoned with them, as a secondary effect. This concept is consistent with the experimental results of Buell, Strauss and Andrus (1) who demonstrated an accumulation of hexose monophosphate in the autolyzing muscle brei of cats in the final stages of adrenal insufficiency. It is a well known fact that animals in severe adrenal insufficiency do not eat voluntarily. It seems possible that inanition may be one means available to the animal of preventing further aggravation of a mounting chemical abnormality in its tissues. It is unfortunate that the quantities of blood available were insufficient for the determination of all of the cations in the serum of each animal. Although the potassium content of the pooled sera was increased above the normal value it gave no hint of the spectacular rise in muscle potassium in animals in adrenal insufficiency. Without tissue analyses this point would have been completely overlooked.

Variations in total base. Another feature of the results was the constancy of the *average value* calculated for the total base in the muscle in all of the groups studied except the group of animals in adrenal insufficiency (table 1). The constancy of this figure is more apparent than real, however, for there were significant variations among individual animals in the same group. For example, the maximum and minimum values in a group of eight normals were 59 and 45 meq., respectively. Variations among individual animals in the other groups were similar. It is recognized that the apparent variations may be greater than, or less than, the actual variations, due to the cumulative or compensatory experimental errors involved in the four separate determinations. Nevertheless, variations *within certain limits* might be expected on the basis of the changing magnitude of the "metabolic potassium" and the limitations of the mechanisms, which were available for the simultaneous adjustment of the other cations. It was only when the total base rose above the value of 62 meq. per 100 grams of dry fat-free muscle that the effects of adrenalectomy were uncompensated and there was serious adrenal insufficiency. It appears that it is a matter of vital importance to the animal to maintain in the skeletal muscle the total base concentration within rather narrow and well-defined limits. With appropriate dosage of sodium chloride or of desoxycorticosterone acetate the effects of adrenalectomy are compensated to a degree which is compatible with life, and it is only in an emergency such as a prolonged fast, vigorous exercise or an infection that such metabolic defects as low liver glycogen, hypoglycemia, and certain altered cation relationships become limiting factors in the animal's survival. In the

absence of specific therapy the potassium content of the muscle soon mounts, and when it reaches a value of 50 meq. per 100 grams of dry, fat-free muscle, effective compensation ceases abruptly.

Relationship of potassium to magnesium. It was reported by Harrison and Darrow (3) that the increase of potassium within the cell in the muscles of rats with adrenal insufficiency "is not associated with any change in the concentration of magnesium which is the other base found within the cells in considerable amounts". Figures 1 and 2 illustrate our experience in this connection. Fortunately, for the sake of simplicity of interpretation, both potassium and magnesium are predominantly cellular elements. It will be seen in figure 1 that the points tend to cluster along two distinct and unrelated curves, the lower curve being fairly well defined, the upper curve less well defined. The points falling on the lower curve all represent potassium values less than 50 meq. per 100 grams of dry, fat-free muscle and include all of the animals in all of the groups studied, except the group of animals in adrenal insufficiency[7]. That is to say, when the adrenal insufficiency was "compensated" by one means or another, the potassium content of the muscle remained below the critical level of 50 meq. and there was an orderly relationship between potassium and magnesium. When the insufficiency was uncompensated, however, the potassium content of the muscle rose. Suddenly, at a value of 50 meq. of potassium, as though there might have been a break in cell membranes due to pressure, there was a break in the linear relationship between potassium and magnesium.

The linear relationship between potassium and magnesium in the compensated animals is seen, in figure 2, to be such that for an increase of 10 meq. of potassium there was, *on an average*, a decrease of 3.5 meq. of magnesium, regardless of the method by which compensation was attained. It transpires that this mechanism of partial (about one-third) compensation of the effects of increases in the "metabolic potassium" by decrease in magnesium is quantitatively the principal adjustment made directly among the cations by means of which the total base is kept within the limits found normally.

Relationship of potassium to sodium. Figure 3 illustrates the fact that the points representing potassium-sodium relationships also fall into two categories, separated by a line representing a potassium value of 50 meq.: 1, the uncompensated condition in which the potassium was high and the relationship between the cations was irregular, and 2, the compensated condition in which the relationship between these cations may be seen in

[7] It will be seen that one point representing an animal in this group fell on the lower curve. In this one case the potassium content of the muscle happened to be below the critical level, and the adrenal insufficiency was compensated, at least for the moment.

more detail in figure 4. Normally muscle potassium is predominantly a cellular element, the quantity of which appears to vary considerably in contrast to muscle sodium which is predominantly an extracellular element, the quantity of which appears to remain fairly constant[8]. It is seen that in figure 4 all of the points fall on or near one of the two curves; *a*, the vertical line representing the normal untreated animals, in which the po-

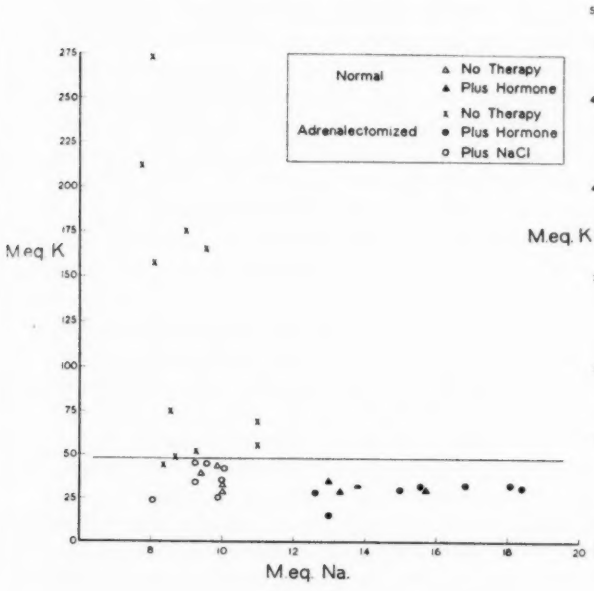


Fig. 3

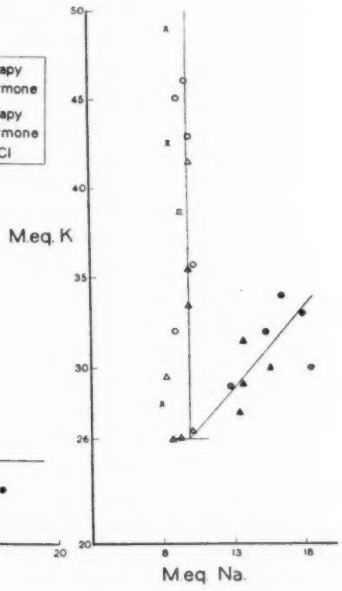


Fig. 4

Fig. 3. Gross relationship between potassium and sodium in the whole skeletal muscles of all of the animals studied, values being reported as milliequivalents of cation per 100 grams of dry fat-free muscle. The hormone used was crystalline synthetic desoxycorticosterone acetate.

Fig. 4. Detailed relationship between potassium and sodium in the whole skeletal muscles of all of the animals which were "compensated," values being reported as milliequivalents of cation per 100 grams of dry fat-free muscle. The hormone used was crystalline synthetic desoxycorticosterone acetate.

tassium appeared to vary independently of the sodium, and *b*, the sloping line in which the potassium and sodium appeared to vary together in nearly equivalent quantities, above a certain minimum level. In group *a* are found the normal untreated animals, the adrenalectomized animals

[8] Heppel (6) however, found that, if rats were deprived of potassium, the sodium content of the muscles was increased two-fold and that most of the sodium in such muscle was intracellular.

which were given sodium chloride, and also two temporarily compensated adrenalectomized animals to which no therapy was given. In these fifteen animals the sodium values varied only between 8.1 and 10.5 meq.

In group *b* are found the animals, both normal and adrenalectomized, which were treated with desoxycorticosterone acetate. The sodium and potassium values of these animals should be considered 1, in comparison with the corresponding values of the same types of animals which had not been treated with the hormone, and 2, in their relationship to each other. First, in the normal animals, treated with hormone, the values for muscle sodium were definitely increased beyond the limit of experimental error and probably beyond an increase in sodium which might have resulted from an increase in the volume of extracellular fluid. There can be little doubt that some sodium ions, in excess of the normal amount, have entered the cells. It will be seen that this effect is qualitatively the same but quantitatively greater in the muscles of the adrenalectomized animals, each given one pellet of the hormone. Larger doses or the same dose over a longer period of time might well have resulted in an even more exaggerated effect[9, 10].

The effect of desoxycorticosterone acetate on muscle potassium in normal animals was equivocal, probably only because it was small; in adrenalectomized animals the effect was dramatic. The average values for muscle potassium for the normal untreated animals was 32 meq. (maximum 42, minimum 26), for the normal treated animals was 30 meq. (maximum 32, minimum 28); for the adrenalectomized untreated animals 118 meq. (maximum 270, minimum 50); for adrenalectomized treated animals 30 meq. (maximum 34, minimum 20). There seems to be no room for doubt that the net effect of the treatment with desoxycorticosterone acetate in the adrenalectomized animals has been 1, the entrance of sodium ions into the muscle cells, and 2, the exit of potassium ions from the muscle cells.

As to the relationship between the sodium and potassium ions in the muscles of all of the animals which were treated with desoxycorticosterone acetate, figure 4 suggests several interesting points. The sloping line, representing this relationship, bisects the vertical line, representing the same relationship in normal muscles, at a point which coincides with the lowest observed value for potassium in normal muscle, which as explained

[9] Recently Miller and Darrow (7) reported decreases in muscle potassium and increases in muscle sodium when normal rats were injected subcutaneously with desoxycorticosterone acetate.

[10] The pellets remained *in situ* for too short a time to permit an accurate determination of the daily dose absorbed by each animal by reweighing the pellets after their removal. By comparison with results obtained in this laboratory (8) with dogs and patients, it may be assumed that approximately 0.3 mgm. of the hormone was absorbed daily by each rat. This dose is equivalent to about 0.5 mgm. injected in oil.

above, has been regarded as a basal value. Furthermore, at least in the few experiments at hand, the sum of the sodium and potassium values seen on the sloping line is in no instance greater than the largest value for "metabolic potassium" in the normal animals. Also, the slope of this line indicates that for values above the basal condition approximately equivalent amounts of sodium and potassium are involved.

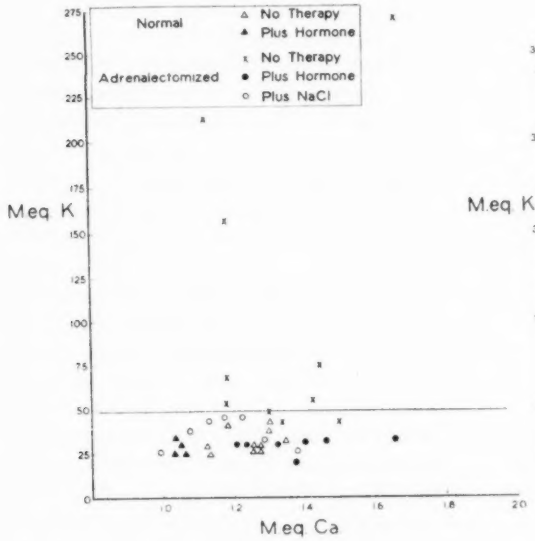


Fig. 5

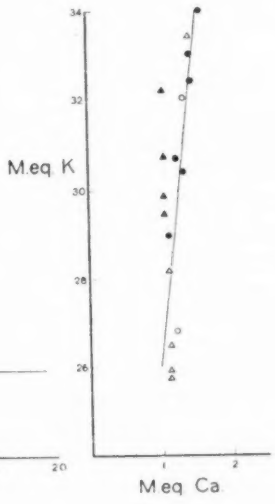


Fig. 6

Fig. 5. Gross relationship between potassium and calcium in the whole skeletal muscles of all of the animals studied, values being reported as milliequivalents of cation per 100 grams of dry fat-free muscle. The hormone used was crystalline synthetic desoxycorticosterone acetate.

Fig. 6. Detailed relationship between potassium and calcium in the whole skeletal muscles of the adrenalectomized animals which had been treated with desoxycorticosterone acetate.

Relationship of potassium to calcium. In our earlier work (1936) a higher calcium content was found in the pooled muscles from several four-hour fasted rats which were in the final stages of adrenal insufficiency, than in the pooled muscles from normal animals. More recently, when the muscles of individual unfasted animals in a milder degree of adrenal insufficiency were analyzed, as is shown in figure 5, high calcium values were observed occasionally but there was no demonstrable correlation with the high potassium values. Even when the insufficiency was controlled, i.e., when the potassium value was below 50 meq., the calcium appeared to

vary almost independently of the potassium. Failure to demonstrate a clean-cut correlation was to be expected, however, because the total quantity of calcium present in muscle was so small as to make its precise determination difficult and the maximum variation among all of the animals studied in this series was less than 1 meq. of calcium per 100 grams of dry, fat-free muscle. The picture is further complicated by the fact that as much as one-fourth of the total calcium in normal muscle is extracellular, a distribution which might mask trends which would otherwise be apparent. Only in the muscles of the adrenalectomized animals receiving desoxycorticosterone acetate but not in the muscles of normal animals, similarly treated, was there a suggested linear correlation between the potassium and calcium values (fig. 6). If future work proves this correlation to be real, it may possibly be more easily demonstrable in the adrenalectomized animals than in the normal, both receiving the hormone, because of the greater permeability of the cell membranes to calcium (and sodium) in the former and also to the smaller amounts of calcium in the serum and extracellular fluid of the former. In any event, for a rise of 10 meq. of potassium above the basal level, there was an apparent corresponding rise of about 0.6 meq. of calcium. The magnitude of the changes in calcium are such as to rule out the importance of these changes quantitatively in the maintenance of the normal value for total base.

General considerations. These experiments suggest new points of view on the puzzling and conflicting data in the literature concerning the part played by potassium not only in adrenal insufficiency but also in normal carbohydrate metabolism. A brief statement of the pertinent facts and theories which are based on good experimental evidence, is presented.

Whenever dehydration occurs, potassium tends to rise in the serum due to the inability of the kidney to excrete it. The total quantity of sodium available to the animal is an important factor in determining the degree of hydration and obviously will be influenced by *a*, the intake of sodium; *b*, the loss due to excretion by the kidney; *c*, the loss due to increased excretion by the intestine, (as in obstruction, diarrhea, etc.); *d*, the loss due directly to hemorrhage or indirectly to withdrawal of fluids into the tissues (as in shock, edema, etc.); *e*, the loss into the ascitic fluid after the experimental injection of glucose into the peritoneum. In all these cases where a decrease occurs in the total sodium content of the circulating fluids, an increase in the serum potassium may be expected and has been observed.

In normal carbohydrate metabolism serum potassium and sugar appear to rise and fall together, probably because potassium hexose phosphate is formed in the muscle cells. The amounts of hexose phosphate have been shown experimentally to be increased as the result of treatment with either insulin or epinephrin (9). On this line of reasoning, a low serum potassium would be expected (and has been observed) at appropriate time

intervals after the administration of either insulin or epinephrin. Lactic acid is formed as the end product of anaerobic metabolism and carbon dioxide and water as the end products of aerobic metabolism. These products escape from the cells, releasing potassium with them, and the potassium content of the serum is increased. This, in our opinion, may be one factor (together with potassium shifts resulting from anoxemia) which explains the high potassium content of the serum after exercise. Since the normal muscle cells contain all four cations, at least certain parts of the cell must have a degree of permeability to all four of them. There is little known definitely about the distribution of the cations in the cell compartments.

With this background, points suggested by these experiments may be discussed:

The muscle cells must be considered as an effective temporary reservoir for storing potassium and removing it from the serum, because of the large values found for the "metabolic potassium" and the large mass of muscle tissue in the animal. The total base of the muscle cells is kept within narrower limits than would otherwise be possible by a simultaneous though not equivalent shift of magnesium out of the muscle cells. It does not appear from the experimental evidence whether the extraordinary increase in muscle potassium in adrenal insufficiency is a primary effect, resulting from a lack of the adrenal cortex, or a secondary effect due to the retention of potassium ions in the cell to neutralize electrically some anions which may be accumulating in the cells in abnormal amounts. The latter possibility is consistent with the observations of Buell, Strauss and Andrus (1) who found *in the muscle brei* of adrenalectomized cats *in extremis* the formation of smaller than normal amounts of lactate (a permeable ion) and larger than normal amounts of hexose phosphate (an impermeable ion). These departures from the normal course of carbohydrate metabolism might be expected to be of greater magnitude in whole muscle or in muscle preparations in which the cell structure was maintained intact than in muscle brei. Interpreted in the light of the theory of Conway and Boyle (5), these facts suggest, but do not prove, that the rise in muscle potassium in adrenal insufficiency is an effect secondary to an error in the anaerobic phase of carbohydrate metabolism. If this is true the beneficial effect in the muscle of potent extracts of the whole adrenal cortex would be due to a correction of the abnormalities in the production of lactate. This hypothesis affords an explanation for the observations described by Kendall et al. (10) as follows: "The results of the injections of glucose . . . indicate that in the absence of cortin or adequate amounts of sodium and chloride the experimental animal becomes highly sensitive to potassium. It therefore seems probable that cortin modifies the rate of some chemical change which involves potassium".

Since the effect of desoxycorticosterone acetate on carbohydrate me-

tabolism is reported to be slight (11) its beneficial effect in compensating for the insufficiency following removal of the adrenal glands may be interpreted as being due to an alleviation of certain effects, rather than the cause, of the insufficiency. On the assumption that the effect of this hormone *in the muscle* is to increase the permeability of the cell membrane (the size of the pores, according to Conway and Boyle) the hexose phosphate anions which formerly accumulated in the cells and retained potassium ions with them might now escape and liberate the potassium cations with which they were associated. As a corollary the sodium ions (which are larger than potassium ions) would now be expected both to enter and to leave the cell indiscriminately with the potassium ions as demanded by the needs for "metabolic potassium", the laws of electrical neutrality, etc. (fig. 4). In this way not only would the tendency toward hypertonicity of the muscle cells in adrenal insufficiency be relieved thus aiding normal hydration of the tissues, but the muscle cells would be freed from the pharmacological effects of high concentrations of potassium ions. (They would, however, be submitted to larger quantities of sodium ions, and the potassium to sodium ratio would be lowered.) The potassium which was released from the muscle cells would temporarily increase the potassium content of the serum, but would readily be excreted in the urine, giving the well known potassium diuresis which occurs early in association with the treatment with desoxycorticosterone acetate (12). There have been reports in the literature (13) of periodic weakness produced in dogs by long continued overdosage with desoxycorticosterone, which was characterized by low serum potassium values and was alleviated by potassium salts and also (14) of transient paralysis and muscular weakness in a patient suffering from Addison's disease, treated daily with 25 mgm. of the hormone, following intravenous injections of sodium chloride and dextrose solutions before and after a nephrectomy. The ratio of potassium to sodium in the muscles, therefore, appears to be a matter not only of physiological interest but also of clinical importance.

The mode of action of sodium chloride in compensating for the specific effects of adrenal insufficiency in the muscle is not clear. Its mode of action on the kidney seems to be simple enough; by making good the loss of sodium and chloride it prevents the depletion of these elements in the plasma, and thus favors normal hydration, normal kidney function and normal serum potassium levels. How it attains the result of keeping the muscle cell potassium within normal limits is not revealed by these experiments. There is no evidence here of increased permeability of the cell membranes; in fact, there appears to be less sodium rather than more, inside the muscle cells. The fact remains however, that treatment with sodium chloride has in some way prevented the increase in muscle potassium which would have occurred in its absence.

The immediate causes of the low blood sugar in adrenal insufficiency are to be sought *a*, in the liver, where there is an impaired ability to synthesize glycogen either from d. lactic acid (2) or from certain metabolically equivalent compounds, such as pyruvic (11) acid which may be secondarily formed from other metabolites arising from protein catabolism, and *b*, in the muscles where there is an impaired ability to form lactic acid (1). A vicious cycle is instituted with respect to the carbohydrate cycle. The injection of intravenous glucose into an animal in acute adrenal insufficiency might benefit the condition not only by relieving the hypoglycemia directly but also by providing a source for the synthesis of liver glycogen (15). Unless some provision were also made for relieving the accumulation of potassium salts already present in the muscle (such as intravenous salt, cortical extract, etc.) this added glucose would take potassium with it into the muscle cells and further aggravate a situation which was already bad. These facts explain the common observation of the effect of intravenous glucose in the treatment of patients suffering from Addison's disease, of a temporary relief of the symptoms, followed shortly by the precipitation of a crisis.

Potassium accumulates in the serum because of *a*, failure on the part of the kidney to excrete it readily because of the dehydration; *b*, failure on the part of the liver to deposit it, as is usually done at least in small quantities, when glycogen is synthesized (16), and *c*, failure on the part of the muscles to take up additional quantities of potassium ions readily against the high gradient of potassium ions already present in the muscle cells. All of these factors act together to institute a vicious cycle with respect to potassium. The result is that in adrenal insufficiency the blood sugar remains low and the plasma potassium remains high in contrast to the normal condition, where they vary together.

These considerations explain why potassium is abnormally toxic in adrenal insufficiency.

It becomes apparent that it is difficult, if not impossible, to dissociate completely the errors in carbohydrate metabolism in adrenal insufficiency from those in electrolyte balance since normal carbohydrate metabolism is intimately associated with changes in the distribution of certain electrolytes. When adrenalectomized animals are "kept in good condition" by one means or another, in order better to study the errors inherent in adrenal insufficiency *per se* unknown factors may unwittingly be introduced which may alter the problem and may thus confuse the issue instead of clarifying it.

SUMMARY AND CONCLUSIONS

1. The skeletal muscles of individual unfasted male rats have been analyzed for moisture, lipid, sodium, potassium, calcium and magnesium.

The animals were studied in the following conditions: 1, normal with no therapy; 2, normal treated with desoxycorticosterone acetate; 3, adrenalectomized treated with sodium chloride; 4, adrenalectomized treated with desoxycorticosterone acetate, and 5, untreated adrenalectomized in varying degrees of insufficiency. Pooled sera from individual animals in each of these groups were analyzed for sodium, potassium, calcium, chloride and non-protein-nitrogen.

2. The potassium content of the muscles of normal unfasted animals varied widely, probably in relation to the demands of carbohydrate metabolism. The potassium content of the muscles of animals in adrenal insufficiency was very high and very variable. This point has been discussed.

3. The average total base of the muscles of all of the groups was remarkably constant regardless of the method by means of which compensation was attained, with the exception of the group in adrenal insufficiency. There was considerable variation in total base among individual animals in the same group.

4. The relationships between muscle potassium and the other cations have been described. Below the critical potassium level there was an orderly relationship between potassium and the other ions. Above this level, i.e., in adrenal insufficiency, this relationship was abruptly changed.

5. Below the critical potassium level, for every 10 meq. of potassium gained by the muscle, on the average, 3.5 meq. of magnesium were lost.

6. Desoxycorticosterone acetate, when administered under the conditions described, caused the entrance of small quantities of sodium into the muscle cells of normal animals and of larger quantities of sodium (and perhaps of a little calcium) into the muscle cells of the adrenalectomized animals. This hormone prevented the rise in muscle potassium which would have occurred in the adrenalectomized animals in its absence. A possible mechanism for this effect is discussed.

7. Sodium chloride likewise prevented the rise in muscle potassium which would have occurred in its absence in the adrenalectomized animals. There was no evidence of increased permeability of the muscle cells in this group of animals.

8. The probable importance of the ratio of potassium to sodium in the muscle cell has been pointed out.

9. The correlation of these new observations in the interpretation of old problems associated with both adrenal insufficiency and carbohydrate metabolism has been discussed.

REFERENCES

(1) Buell, M. V., M. B. Strauss and E. C. Andrus. J. Biol. Chem. **98:** 645, 1932.
(2) Buell, M. V., I. A. Anderson and M. B. Strauss. This Journal **116:** 274, 1936.
(3) Harrison, H. E. and D. C. Darrow. J. Clin. Investigation **17:** 77, 1938.

(4) Buell, M. V. J. Biol. Chem. **130:** 357, 1939.
(5) Conway, E. J. and P. J. Boyle. Nature **144:** 709, 1939.
(6) Heppel, L. A. This Journal **127:** 385, 1939.
(7) Miller, H.C. and D. C. Darrow. This Journal **132:** 801, 1941.
(8) Thorn, G. W., L. L. Engel and H. Eisenberg. Bull. Johns Hopkins Hosp. **64:** 155, 1939.
(9) Cori, C. F. and G. T. Cori. J. Biol. Chem. **94:** 581, 1931, 32.
(10) Kendall, E. C., E. V. Flock, J. L. Bollman and F. C. Mann. J. Biol. Chem. **126:** 697, 1938.
(11) Lewis, R. A., D. Kuhlman, C. Delbue, G. F. Koepf and G. W. Thorn. Endocrinology **27:** 971, 1940.
(12) Thorn, G. W., R. P. Howard and K. Emerson, Jr. J. Clin. Investigation **18:** 449, 1939.
(13) Kuhlmann, D., C. Ragan, J. W. Ferrebee, D. W. Atchley and R. F. Loeb. Science **90:** 496, 1939.
(14) Thorn, G. W. and W. M. Firor. J. A. M. A. **114:** 2517, 1940.
(15) Deuel, H. J., Jr., L. F. Hallman, S. Murray and L. T. Samuels. J. Biol. Chem. **119:** 607, 1937.
(16) Fenn, W. O. J. Biol. Chem. **128:** 297, 1939.

THE RENAL EXCRETION OF AN ANTIDIURETIC SUBSTANCE BY THE DOG

KENDRICK HARE, ROBERT C. HICKEY AND RUTH S. HARE

From the Department of Anatomy, Cornell University Medical College, New York City

Accepted for publication June 28, 1941

The normal water exchange of most laboratory mammals depends upon the presence of the pars nervosa of the pituitary. Transection of the pituitary stalk or destruction of the supra-optic nuclei in the hypothalamus results in atrophy of the pars nervosa and an increase in water exchange; administration of extracts of the pars nervosa will then reduce the water exchange to its normal level. While these facts have often been demonstrated, the rôle of the pars nervosa in the regulation of water exchange in the intact animal is only vaguely defined.

Gilman and Goodman (1) discovered an antidiuretic material in the urine of normal dehydrated rats. They concluded that this substance was of pituitary origin since it was not found in the urine of dehydrated rats after hypophysectomy. Walker (2), in a more comprehensive investigation, tried to find "an antidiuretic hormone in body fluids in concentrations which vary with physiological variations in urine volume". Although some of his experiments confirmed Gilman and Goodman, discrepancies in others caused him to doubt their conclusions. Assays by Ingram, Ladd, and Benbow (3) of urine from dehydrated normal and diabetes insipidus cats provide strong evidence that the excretion of an antidiuretic material by the cat depends upon the presence of the pars nervosa.

We have assayed the urine, blood and pars nervosa of normal and diabetes insipidus dogs after dehydration. The method of assay (4) differs from that used by other investigators. As a test animal we have used the diabetes insipidus dog which will maintain a diuresis for several hours. The criterion of antidiuresis is not simply a decrease in urine flow, but an increase in urine concentration. Since urine flow is the resultant of two independent functions, glomerular filtration and tubular reabsorption of water, it is essential in evaluating antidiuretic effects to consider each separately. This is especially important since Burgess, Harvey and Marshall (5) have shown that an extract of the pars nervosa effects an antidiuresis by increasing tubular reabsorption of water.

The volume of glomerular filtrate was measured by the renal clearance of creatinine, since there is good evidence that exogenous creatinine is excreted

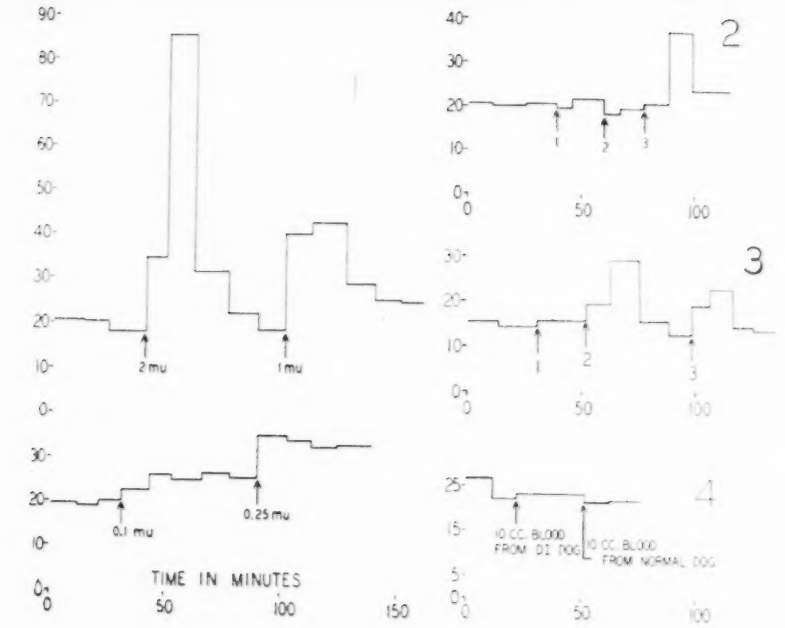
solely by glomerular filtration in the dog (6, 7). The creatinine U/P ratio, or the number of cubic centimeters of plasma required to produce 1 cc. of urine, was used as a measure of water reabsorption. One-tenth of a milliunit of Pitressin (Parke-Davis) produced a measurable increase in creatinine U/P ratio (fig. 1).

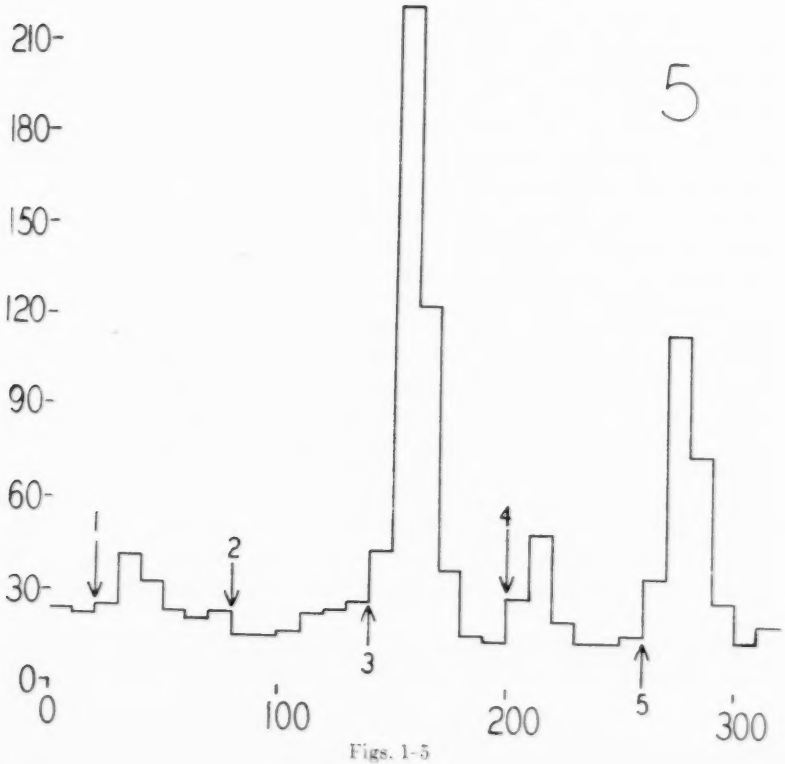
Intravenous injection of the material to be assayed was used for two reasons. First, the rates of absorption have been shown to vary greatly after subcutaneous injection (8), and in our assays duration of the antidiuresis was an important part of the response. Second, since large volumes of fluid may be given intravenously, it was unnecessary to concentrate the urine by procedures which might alter its constituents (1, 2, 3, 9). In some assays as much as 100 cc. of fresh untreated urine were injected.

METHOD. Five grams of creatinine in 100 cc. of water were given by stomach tube to the test animal (a diabetes insipidus dog) about 2 hours before the assay and about 18 hours after feeding. The dogs, all females, were accustomed to catheterization and venipunture and lay quietly on the board. Blood samples were collected at one hour intervals, centrifuged immediately, and duplicate aliquots of plasma precipitated with tungstic acid. The bladder was emptied by washing out with air and urines collected at carefully timed intervals, usually of 10 minutes' duration. Errors in volume measurement were minimized by collecting the urines in volumetric flasks which were then filled to the mark with distilled water from a burette. At least two control clearances were run in every experiment before any injections were given. Creatinine concentrations were determined by the alkaline picrate method, and the clearance and U/P ratio, calculated. The accuracy of the method was improved by using a photoelectric colorimeter and by developing the color of the solutions in a room maintained at a constant temperature.

Donor animals were normal dogs and dogs which had a permanent polyuria as the result of pituitary stalk section. Blood and urine samples for assay were collected from these dogs after a water deprivation of 2 to 4 days. The dogs were catheterized and the bladder washed out with 0.9 per cent saline. After an interval of 15 minutes to 2½ hours, the urine was collected, the bladder again washed with saline and the washing added to the urine. The specimens thus collected were diluted to the same volume and injected intravenously into the test animal. In a few cases, blood samples for assay were also collected. Ten or twenty cubic centimeters of blood were withdrawn from the external jugular vein through a 15 gauge needle and immediately injected into the test animal. No anticoagulant was used.

In one experiment the donor dogs were killed immediately after the urine collection, and the pars nervosa of the pituitaries removed. Each


2 mu
1 mu
0.1 mu
0.25 mu
TIME IN MINUTES
2
3
4
10 CC. BLOOD FROM DI DOG
10 CC. BLOOD FROM NORMAL DOG


5

Figs. 1-5

gland was thoroughly macerated with sea sand in saline and an aliquot of this extract assayed in the same manner as the urines. Assays were also done on urine samples collected from diabetes insipidus dogs before and after injection of fresh saline extracts of posterior lobes.

RESULTS. The urine from dehydrated normal dogs invariably caused an antidiuresis when injected into a diabetes insipidus dog. This antidiuresis was the result of an increase in the concentration of the urine as expressed by the creatinine U/P ratio (figs. 2 and 3). The urine from dehydrated diabetes insipidus dogs never produced an antidiuresis; in 6 out of 7 cases, it was followed by a slight diuresis. The blood from normal and diabetes insipidus dogs never produced any effect on creatinine clearance or U/P ratio in the test animal (fig. 4). This finding is apparently contradictory to Melville's results (10). The discrepancy may be accounted for in three ways: Melville made an extract of the blood; he used a larger volume (50 cc.); and since he did not assay the blood of the hypophysectomized dog, the substance he found may be not be of pituitary origin. Since 0.1 milliunit of Pitressin was easily detected by our method, the blood assayed in this experiment must have contained less than the equivalent of that amount in 10 cc. When the posterior lobes of the donor dogs were assayed, the response of 1/10 of the diabetes insipidus gland was less than half of the smaller response to 1/500 of the normal posterior lobe (fig. 5). The antidiuretic potency of the diabetes insipidus posterior lobe was therefore less than 2 per cent of the normal.

Fig. 1. Two experiments were done on a diabetes insipidus dog to determine the threshold dose of Pitressin. The upper line indicates the responses to 2 and 1 milliunit doses; the lower line, the responses to $\frac{1}{10}$ and $\frac{1}{4}$ milliunit doses. In all figures the ordinate is time in minutes; the abscissa, creatinine U/P ratio.

Fig. 2. Assays of 30 minute urine specimens from 2 diabetes insipidus and 1 normal dog deprived of drinking water for 97 hours. All specimens were diluted to 20 cc. with isotonic saline and injected intravenously. The urines from the polyuric dogs were injected at the first and second arrows; the urine from the normal dog at the third arrow. Only the last caused an antidiuresis.

Fig. 3. Assay of 30 minute urine specimens from 1 diabetes insipidus and 2 normal dogs dehydrated for 47 hours. Each urine was diluted to 20 cc. with isotonic saline and injected intravenously. The diabetes insipidus urine was injected at the first arrow; the urine from the normal dogs at the second and third arrows.

Fig. 4. Assay of blood from normal and diabetes insipidus dogs. Neither sample of blood contained a detectable amount of antidiuretic substance.

Fig. 5. Assay of urines and pituitaries of a normal and of a diabetes insipidus dog. After the dogs had gone without water for 68 hours, $2\frac{1}{2}$ hour urine specimens were collected from each. The urine from the normal dog was injected at the first arrow; the urine from the diabetes insipidus dog at the second arrow. After the urine collection, the dogs were killed, their pituitaries removed and macerated in saline. At the third arrow $\frac{5}{100}$ of the normal dog posterior lobe was injected; at the fourth arrow $\frac{1}{10}$ of the posterior lobe of the diabetes insipidus dog was injected. The fifth injection is a repetition of the third.

Although urine samples from the untreated diabetes insipidus dog had no antidiuretic effect, urine samples taken from the same dog after the injection of a saline extract of pars nervosa had a marked antidiuretic effect. This result shows that the kidney of the diabetes insipidus dog, like that of the normal rabbit (11) is capable of excreting an antidiuretic substance.

CONCLUSIONS

1. The urine of the dehydrated normal dog contains an antidiuretic substance; the urine of the dehydrated diabetes insipidus dog contains none.

2. If there is any antidiuretic substance in the circulating blood, it is present in concentrations equivalent to less than one milliunit of Pitressin per 100 cc.

3. The kidney of the diabetes insipidus dog is capable of excreting an antidiuretic substance when the material is administered by intravenous injection.

4. The absence of an antidiuretic substance from the urine of the dehydrated diabetes insipidus dog is associated with an enormously diminished antidiuretic content of the pars nervosa.

REFERENCES

(1) Gilman, A. and L. Goodman. J. Physiol. **90:** 113, 1937.
(2) Walker, A. M. This Journal **127:** 519, 1939.
(3) Ingram, W. R., L. Ladd and J. T. Benbow. This Journal **127:** 544, 1939.
(4) Hare, K. Proc. Am. Physiol. Soc. p. 79, 1940.
(5) Burgess, W. W., A. M. Harvey and E. K. Marshall. J. Pharmacol. and Exper. Therap. **49:** 237, 1933.
(6) White, H. L. and B. Monaghan. This Journal **106:** 16, 1933.
(7) Van Slyke, D. D., A. Hiller and B. F. Miller. This Journal **113:** 611, 1935.
(8) Noble, R. L., H. Rinderknecht and P. C. Williams. J. Physiol. **96:** 293, 1939.
(9) Martin, S. J., H. C. Herrlich and J. F. Fazekas. This Journal **127:** 51, 1939.
(10) Melville, K. I. J. Exper. Med. **65:** 415, 1937.
(11) Heller, H. J. Physiol. **89:** 81, 1937.

MODIFICATION OF THE PANCREATIC RESPONSE TO SECRETIN BY URINE AND URINE CONCENTRATES

HARRY GREENGARD, I. F. STEIN, JR. AND A. C. IVY

From the Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago

Accepted for publication June 28, 1941

We have noted (1) that blood serum has the property of inactivating secretin and that such inactivation is on an enzymic basis; furthermore, that although secretin is inactivated by gastric and pancreatic juice and by crude extracts of pepsin and trypsin, it is unaffected by these enzymes in crystalline form. On the strength of these findings it was postulated that the ineffectiveness of orally administered secretin was due to the presence of secretinase in the gastrointestinal secretions which destroy the hormone before it can be absorbed. Hence it appeared not improbable that secretinase was rather widely distributed in the body and that the question of its occurrence in the urine merited investigation. Moreover, a demonstration of secretinase in the urine would afford a logical explanation for the circumstance that secretin has never been found in urine or urine concentrates.

Consequently a series of experiments was conducted with a view to answering this question. In the course of this work, it became apparent that when the animals were given a large amount of urine or urine concentrate the pancreas became increasingly refractory to control injections of secretin; the agent causing this refractoriness appeared to be present in both unboiled and boiled material. This indicated the presence in the urine of a thermostable substance which inhibits the secretory activity of the pancreas. That such a circumstance is not unlikely is indicated by the studies of several investigators (2–6) who have demonstrated that the urine contains a gastric secretory depressant.

In other words, we considered the possibilities that the urine contains substances which may act on the hormone itself and on the end-organ excited by the hormone. These possibilities were examined in the experiments detailed below.

EXPERIMENTAL. 1. *Materials.* Human urine served as the source material. It was used in the following forms: untreated; vacuum distilled and dialyzed; the precipitate obtained by the addition of 10 volumes of acetone to vacuum distilled dialyzed urine; and the benzoic acid adsorbate

according to the method of Katzmann and Doisy (7) suspended in water, boiled, filtered, and precipitated with acetone. Various other reagents were tried and found of no use. The dry preparations were injected in 100-mgm. doses.

2. *Secretinase in the urine.* The animal preparations and secretin concentrates were the same as those we employed in our previous studies (1). The presence of secretinase was tested by incubating freshly voided human urine with the standard amount (1 mgm.) of secretin concentrate and comparing the response to that elicited by a standard dose of secretin and by a mixture of secretin with boiled and cooled urine similarly incubated. The urine concentrates were assayed for secretinase in a similar manner.

3. *Pancreas inhibitor in the urine.* The same animal preparations were used except that in some dogs the secretin was injected in dilute solution into the femoral vein by a Woodyatt pump at a very slow and constant rate (0.1 mgm. per min.) in order to stimulate a slow and regular flow of pancreatic juice from the cannulated duct. When such a flow was established one of the urine concentrates was injected and pancreatic flow recorded for one to two hours thereafter. In most of the dogs the pancreatic response to the standard dose of secretin was ascertained, following which the urine concentrate was injected. At subsequent intervals the secretin administration was repeated and the pancreatic response noted with reference to the control.

RESULTS. 1. *Urinary secretinase.* In all cases in which secretin was incubated with freshly voided normal urine a definite inactivation of the secretin occurred, whereas there was no inactivation when previously boiled and cooled urine was used. The results are listed in table 1.

2. *Pancreas inhibitor.* Indication of the presence of this agent was obtained when *urine concentrates* were incubated with secretin. It was observed that there was an apparent inactivation by both boiled and unboiled urine concentrates, and furthermore, the response to control injections was definitely less for some time after they were administered. The data illustrating these findings are listed in table 2.

When the urine concentrates were injected intravenously into dogs secreting at a uniform rate in response to continuous secretin injection, a retardation of the secretion was noted. Such retardation was marked when the secretory rate of the pancreas was slow, and was minimal when the gland secreted at a rapid rate. Illustrative records are shown in figure 1. Throughout these experiments complications were encountered in about half the dogs in the form of a drop in blood pressure which was mild in degree (30 mm. Hg or less), gradual in onset, and up to 20 minutes in duration. It was to avoid this that the procedure was altered to that of standardizing the animal to a single dose of secretin, then injecting the urine concentrate, waiting for recovery from any consequent hypotensive

TABLE 1

Enzymic inactivation of secretin: alterations in response to a standard dose of secretin following incubation with 5 cc. of urine

DOG	PROCEDURE	INCUBATION TIME	RESPONSE (DROPS)	CONTROL (DROPS)
		hours		
1	Secretin + urine	4	11	27
2	Secretin + urine	3	14	31
	Secretin + boiled urine	3	26	
3	Secretin + urine	3	7	20
	Secretin + boiled urine	3	21	
	Secretin + urine	5½	6	29
	Secretin + boiled urine	5½	27	
4	Secretin + dialyzed urine	4	0	35
	Secretin + boiled dial	4	24	
5	Secretin + urine	4	16	33
	Secretin + boiled urine	2½	33	
	Secretin + urine	4½	14	36
	Secretin + boiled urine	4½	31	
6	Secretin + urine	24	7	75
	Secretin + boiled urine	24	45	
7	Secretin + urine	3	6	28
	Secretin + boiled urine	3	26	
8	Secretin + urine	4	0	12
	Secretin + boiled urine	4	10	
9	Secretin + urine	5	36	84
	Secretin + boiled urine	5	78	
10	Secretin + urine	4	12	32
	Secretin + boiled urine	4	27	

TABLE 2

Refractoriness to secretin after injection of urine concentrates

INJECTION	RESPONSE (DROPS)	
	Dog 11	Dog 12
Control (1 mgm.)	42	37
Secretin + dialyzed urine	0	3
Secretin + boiled dialyzed urine	9	11
Secretin + acetone ppt. from dialyzed urine	0	0
Secretin + boiled acetone ppt. from dialyzed urine	0	6
Control	17	8
Control after 1 hour		37
Control 1 minute after acetone ppt.		1

effect, and finally repeating the standard secretin injection at intervals. In all animals administration of the urine concentrate was followed by a relatively refractory state of the pancreas, which was maximal in 15 to 20 minutes, gradually diminished, and had usually disappeared in two hours. The data are listed in table 3.

Discussion. The results of the assays for secretinase in the urine are self-explanatory. It is apparent from the data obtained that there exists

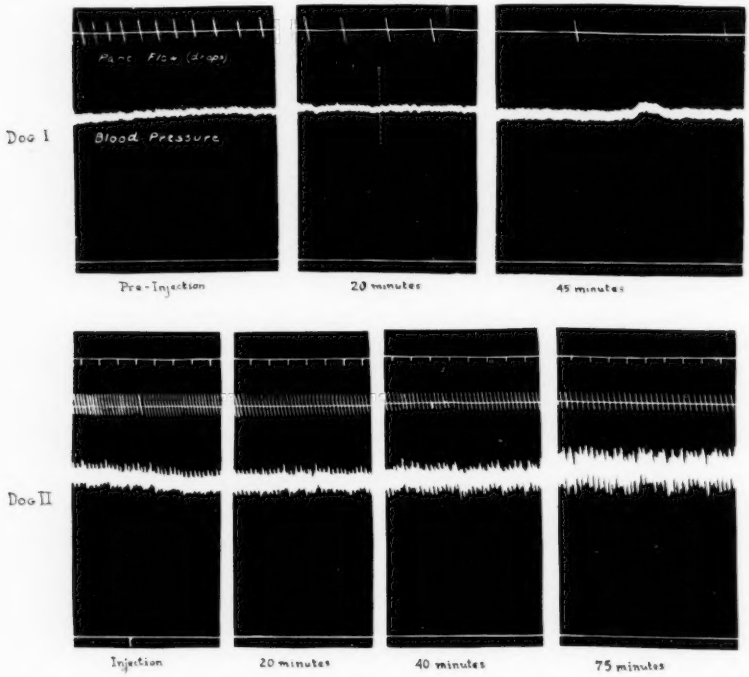


Fig. 1. Pancreatic secretion in response to continuous injection of secretin, 0.1 mgm. per minute, showing retardation of secretion following intravenous injection of urine concentrate.

in the urine a thermolabile substance which inactivates secretin. Whether this enzyme is identical with the secretinase demonstrated to exist in blood serum is problematical. The other principle demonstrated to be present in the urine is heat-stable, non-diffusible, and exerts its effect directly on the pancreas. Whether this effect is a direct inhibition of the secretory activity of the pancreatic acinar cells, or whether it is primarily vascular in nature, cannot be answered until a satisfactory method is developed for measuring blood flow through the pancreas.

Two circumstances argue against the likelihood of a vascular mechanism. In the first place, the hypotensive effect of the urine concentrates was inconstant and in half the animals was entirely absent. Secondly, in three of the animals which had evidenced a fall in blood pressure, the hypotensive effect was controlled by injections of peptone. To do this, a sufficient interval was allowed to elapse after administration of the urine concentrate until the pancreas had regained its normal activity, after which 2 cc. of 10 per cent Witte's peptone was injected. Following recovery, which took place in 5 to 10 minutes, the secretin injection was repeated and the response found to be identical with that obtained prior to the peptone injection. It was concluded from these observations that a transitory fall

TABLE 3

Pancreatic response in drops to a standard dose of secretin before and after injection of a urine concentrate

DOG NO.	BEFORE		AFTER										
	Control response		Time in minutes										
			10	20	30	40	50	60	70	80	90	100	110	120
13	23	23	14	15	5			8				10		17*
14	21	20	17	12		14				18	17			
	17		13	9			13			13				
15	27	25	13	13		15			21		24			
16	36		2		8		13	24						
17	30			15		19	21		28					
18	31	32			24	20		18	28		31			
		31		18	15			16					25	
19	13	13		1							16		25	25
20	32				13		19		25		32		32	
21	46	48	32		18		16		24		35	40		42
21	50		32		26		15	18			35			44

* Four hours.

in blood pressure *per se* has no lasting effect on pancreatic secretory activity.

The effect on pancreatic secretion cannot be a result of contamination with pyrogen, since the urine concentrate was prepared under precautions designed to eliminate bacterial growth and was free of demonstrable pyrogenic effect. A by-product very high in pyrogen, supplied us by Doctor Gray, failed to show any inhibitory effect on the pancreas.

The agent in the urine responsible for the vaso-depressor effect of the concentrates is obscure. The only material which has been characterized as having such an action is callicrein (8), which is heat-labile and therefore could not be the factor in the urine concentrates used in this work, which had been boiled in the process of preparation. Moreover, the hypotensive

action of callicrein is rapid in onset and brief in duration. Wollheim and Lange (9) and Wollheim (10) have noted a vasodepressor activity in extracts from urine and from posterior lobe of the hypophysis which is very similar to that noted in this work, and which they ascribe to a thermostable substance termed "depressan" or "detonin".

The thermostable pancreatic inhibitor demonstrated to exist in the urine has been called *uropancreatone*. This is in conformity with the name urogastrone applied to the gastric inhibitory agent described by Gray *et al.* (loc. cit.). The present findings suggest the interesting possibility that the urine contains one or more principles which may regulate the production of several of the digestive secretions. The presence of such bodies in the urine obviously denotes their excretion; their original source remains to be established.

SUMMARY AND CONCLUSIONS

Assays of human urine for secretinase activity have been conducted, and the presence of such an enzyme in the urine demonstrated. In addition, the urine has been shown to contain a thermostable principle which directly affects the secretory activity of the pancreas, and this is termed uropancreatone.

REFERENCES

(1) GREENGARD, STEIN AND IVY. This Journal **133:** 121, 1941.
(2) GRAY, WIECZOROWSKI AND IVY. Science **89:** 489, 1939.
(3) GRAY, CULMER, WIECZOROWSKI AND ADKISON. Proc. Soc. Exper. Biol. and Med. **43:** 225, 1940.
(4) GRAY, WIECZOROWSKI, CULMER AND ADKISON. This Journal **129:** 589, 1940.
(5) FRIEDMAN, RECKNAGEL, SANDWEISS AND PATTERSON. Proc. Soc. Exper. Biol. and Med. **41:** 509, 1939.
(6) NECHELES, HANKE AND FANTL. Proc. Soc. Exper. Biol. and Med. **42:** 618, 1939.
(7) KATZMANN AND DOISY. J. Biol. Chem. **98:** 739, 1932.
(8) BISCHOFF AND ELLIOTT. J. Biol. Chem. **109:** 419, 1935.
(9) WOLLHEIM AND LANGE. Deutsch. med. Wchschr. **572:** 1932.
(10) WOLLHEIM. Acta Med. Skand. **91:** 1, 1937.

ACTIVITY IN ISOLATED SYMPATHETIC GANGLIA

KENDRICK HARE

From the Department of Anatomy, Cornell University Medical College, New York City

Accepted for publication June 30, 1941

REFLEXES IN SYMPATHETIC GANGLIA. A great variety of sympathetic responses may be obtained when stimuli are applied to afferent fibers from which the impulses are ultimately transmitted to efferent sympathetic neurons. If sensory neurons were present in the sympathetic ganglia, a reflex arc complete in the isolated ganglion would be a possibility. Dogiel (1) described such neurons, but his interpretation has been doubted by Langley (2), Ranson (3) and many others.

The physiological evidence for such a reflex mechanism is just as equivocal as the anatomical evidence, and many of the phenomena first thought to illustrate reflexes in the peripheral sympathetic ganglia have since been interpreted differently. According to Langley (2), "The peripheral neurons connected with a given spinal neuron are not necessarily all in one ganglion. . . They are connected with three, four, or more ganglia. This fact affords explanation of nearly all the 'reflex' actions which have been described as occurring in peripheral ganglia. The nervous impulse set up in one branch of the preganglionic fiber passes to the other branches, so to the peripheral ganglia, and to tissues more or less remote from the point stimulated. These reflexes may be called preganglionic axon reflexes" (pp. 11–12).

Study of the activity of the peripheral sympathetic system has been aided by the use of the galvanic skin reflex. It has been shown that the changes in the conductivity of the skin are most marked over the palmar and plantar surfaces of the hands and feet, and that these changes are effected largely, if not solely, through the sweat glands.

Using this technique, Schwartz (4) studied the sympathetic response in the pads of cats after the dorsal roots of the brachial plexus had been cut, and after the stellate ganglion had been cut off from the central nervous system. He concluded that "changes in the skin resistance in the pad of a cat's forepaw occur in response to reflex activity of the sympathetic nervous system. In the present study it is shown that a certain fraction of these reflex impulses are mediated solely through the stellate ganglion; that is, they are true sympathetic reflexes and require no central connection of the stellate ganglion. The afferent fiber carries impulses

from the blood vessels or deeply lying tissue of the forelimb, through the grey ramus, to its cell of origin in the stellate ganglion. There synaptic relations with the efferent neurons occur".

Magoun, Hare and Ranson (5), studying the effects of cerebellar stimulation upon the contractions of deafferented muscle, prepared some cats with the sensory innervation of the forelimb abolished. The dorsal roots of the fourth cervical through the second thoracic segments were cut according to the technique of Schwartz. Six cats were prepared in this manner, but when they were subjected to a careful sensory examination, it was found that each cat was still sensitive on the deafferented side over the posterior axillary fold, the dorsal surface of the arm, the posterior part of the elbow, and for about a centimeter distal to the elbow along the ulnar side of the forearm. Consequently, a second series of cats was prepared with the dorsal roots cut from the fourth cervical through the fifth thoracic segments. Sensory examination revealed total anesthesia of the affected forelimb. This observation aroused the suspicion that the activity which Schwartz had observed might be due to the stimulation of afferents of cerebro-spinal origin, which activated the sympathetic ganglia through the preganglionic fibers of the ventral roots.

The purpose of the following experiment was to test this possibility. The criterion of sympathetic activity used was a decrease in the resistance of the forepad to the flow of a galvanic current. The procedure of examination was as follows: the cat was fed, and immediately placed in a canvas strait-jacket, which was designed to avoid any interference with the circulation of the forelimbs. The cat was continuously petted by an attendant, and all of the observations reported were made when the cat was quiet. Leads were taken from the center pads with saline-zinc sulphate-zinc electrodes, held in place by strips of dental dam. If recordings were to be made from more than one pad, all the electrodes were fastened on at the beginning of the experiment, and the leads shifted from one to another. The low resistance electrode was usually applied to a region of the tail anesthetized with novocain and incised to eliminate skin resistance. In the examination of cats with deafferented forelimbs, the low resistance electrode was often placed on the anesthetized shoulder; it is essential that this electrode be placed on an anesthetized area, since the pain of application of saturated zinc sulphate solution to a fresh incision would cause such a burst of sympathetic activity, that an additional reflex discharge would be obscured. The resistance of the forepad was measured with a potentiometer (fig. 1) in which a variable resistor was balanced against the resistance of the cat. A change in skin resistance caused a deflection of the galvanometer which was sensitive enough to detect a change of less than one-tenth of one per cent. The resistance was measured between the electrode over the incised skin of the tail or shoulder and the

electrode on one of the forepads; that the greater part of this resistance was offered by the skin of the forepad was shown when a superficial scratch through the skin at this point caused the resistance of the cat to fall from 10–15,000 ohms to 1–2,000 ohms. Visual, auditory, and cutaneous stimuli were used. In a few cases the galvanic skin reflexes were recorded with a Hindle model string galvanometer.

Ten cats with dorsal roots C_1 through T_5 cut on the left side were repeatedly examined for sympathetic responses. Stimulation of the anesthetized limb, even when extremely severe (pinching with hemostats,

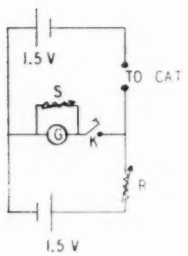


Fig. 1

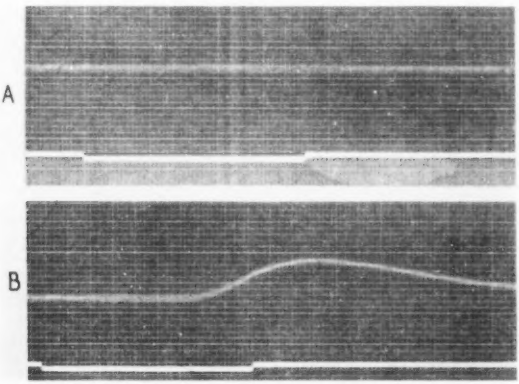


Fig. 2

Fig. 1. Potentiometer for measuring skin resistance. *G*, Leeds and Northrup galvanometer 2420-c.; *S*, shunt; *R*, one megohm decade resistor in steps of 100 ohms; *K*, switch.

Fig. 2. String galvanometer recordings of skin resistance. (Recorded by Dr. Dayton J. Edwards.)

A. Absence of change in left fore pad on crushing the left elbow with heavy forceps.

B. Fall in skin resistance in right pad on pinching the cat's tail.

Lower line indicates onset and duration of stimulus. Dorsal roots C_4–T_5 and ventral roots C_8–T_{10} cut on the left side.

burning with the tip of a hot metal rod) caused no change in the resistance of the forepad of the same or the opposite limb. Flashing a light into the animal's eyes, blowing a whistle, or pinching any normally innervated skin, caused a sharp fall in the resistance of both forepads. Four cats with the left dorsal roots C_1 through T_2 cut were similarly examined, and all gave responses to stimulation of the region of the elbow and the posterior axillary fold of the affected limb.

These results may be given either of two interpretations: first, that the response depended on the presence of afferent nerves in the extremity being stimulated; second, that in the first group there was enough activity

in the sympathetic ganglia, which retained their normal preganglionic innervation, to mask any added reflex. In order to exclude this second and improbable possibility the preganglionic sympathetic paths to the deafferented forelimb were severed. The ventral roots of the left side were cut from T_1 through T_{10}. This deprived the limb of its preganglionic sympathetic innervation but left its sensory innervation intact and caused only a slight deficit of motor nerve supply. This resulted in an increase in resistance of the pad of the affected limb to 10,000–40,000 ohms above the normal level. The resistance was not altered by the most painful stimulation of sensitive areas of the cat's skin, and was therefore considered unaffected by the emotional state of the animal at the time of examination. When the stimulus was made excessively noxious, the cat could not be restrained for proper examination. Even when the stimulus was applied to the left forelimb no decline in resistance could be measured.

To avoid this difficulty and to test for reflex activity of the isolated ganglia under what was thought to be the most ideal of conditions, 3 cats were subjected to the following operation: the dorsal roots from C_4 through T_8 and the ventral roots from C_8 through T_{10} were cut on the left side. Thus, only the postganglionic sympathetic nerves of the limb were left, and not only were the sweat glands in the pad freed from impulses originating elsewhere in the body, but, since the limb was anesthetized, very strongly noxious stimuli could be applied without causing the cat any discomfort. No change in resistance could be produced by any kind of stimulation, even though pressure on the elbow was applied with forceps and large areas of skin on the arm crushed with hemostats. However, if leads were taken from the center pad of the opposite and normal limb, pinching the tail caused a fall in resistance of 2000 ohms, when the original resistance was 9000 ohms. The failure of the left forepad to respond cannot be attributed to the condition of the animal (fig. 2, record). It is attributed to the inability of the ganglia of the sympathetic chain to mediate reflexes when severed from their connections with the central nervous system.

Schwartz (4), however, came to the opposite conclusion. He measured the change in resistance in ten cats with "somatically-deafferented" right forelimbs (p. 597). Deep pressure on the deafferented limb caused a decrease in resistance of the left forepad. The following explanation was offered: "The mechanism causing this response probably consists of afferent sympathetic impulses passing down the right thoracic chain to below the level of the second thoracic; there the impulse can pass through the intact dorsal roots and cross over and upwards to the opposite side" (p. 598). In other words, Schwartz (4) explains a reflex initiated in deafferented tissue by suggesting that the tissue is not completely deafferented. One of the animals in his series was subjected to an additional

operation: the sympathetic chain above and below the right stellate ganglion was resected, and all rami from the ganglion were severed except the gray ramus to the first thoracic nerve. He concluded: "The right forelimb, accordingly, received its sole sympathetic supply from the stellate ganglion through one gray ramus, the ganglion being isolated from all other nervous connections" (p. 598). Reflex changes in resistance in this right forelimb (fig. 3, p. 598) were caused by auditory stimuli or by pinching the tail. This offers excellent evidence that the sweat glands of the right forelimb still retained a connection with the central nervous system. According to Nonidez and Hare (6), the gray rami may contain both pre- and postganglionic nerve fibers. Zuckerman (7) described a fusion of the white and gray rami in the monkey, and Kuntz (8) observed a similar arrangement in man. Langley (9) denied admixture of pre- and postganglionic fibers in the rami communicantes in the cat. In view of this anatomical evidence, it is dangerous to assume that no preganglionic fibers remain after section of all white rami to the sympathetic ganglion. A more dependable procedure for elimination of preganglionics is section of the appropriate ventral spinal roots as our third group of animals showed.

Bolton, Williams and Carmichael (10) studied the vasomotor responses in two patients with cord lesions. In one case the spinal cord caudal to the fifth thoracic segment was inactive, but the dorsal root ganglia and the sympathetic chain were preserved; in the other, a lesion of the cauda equina had destroyed the dorsal and ventral roots caudal to the second lumbar level. In neither case could vasomotor changes in the toes be elicited by stimulation of the anesthetized part of the body although the sympathetic chain ganglia were intact in each case.

Bronk et al. (11) divided a cardiac branch of the stellate ganglion of the cat into two fascicles. All other branches of the ganglion were then severed. Stimulating electrodes were applied to one fascicle of the cardiac nerve, recording electrodes to the other. Stimulation of one fascicle produced no response in the other. This is additional evidence against the idea of a reflex arc within the sympathetic ganglion.

Tonic activity in isolated sympathetic ganglia. Govaerts (12), and Tower and Richter (13) have presented evidence that sympathetic ganglia, when severed from the central nervous system, maintain a tonic or continuous activity, as distinguished from a reflex activity. Govaerts (12) recorded continuous discharges in postganglionic nerves arising from isolated sympathetic ganglia. Bronk et al. (11) failed to confirm Govaerts' findings. Tower and Richter (13) measured the galvanic resistance of the forepads of cats after section of the white rami, and later after removal of the thoracic sympathetic chain on the same side. The first of these operations was considered a preganglionic denervation, the second a postganglionic. Measurements of skin resistance begun before the first opera-

tion and continued after the second, showed that postganglionic denervation caused a greater rise in skin resistance than did section of the white rami to the ganglia. In other words, section of the white rami did not cause a total paralysis of the sympathetic ganglia. Their method of preganglionic denervation, section of the white rami, is subject to the criticisms made in the first part of this paper.

In order to avoid this source of error, we removed the thoracic and cervical sympathetic chain on the right side from six cats, and later cut the left ventral spinal roots from T_1 through T_9. This permitted simul-

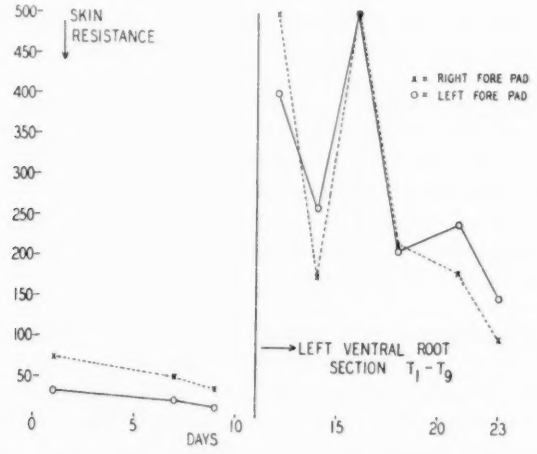


Fig. 3. Skin resistance of a cat after post- and preganglionic sympathectomy. Abscissa: time in days. Ordinate: skin resistance in thousands of ohms.

The right stellate ganglion and thoracic chain were excised two months before these observations were begun. Before the ventral root section on the 11th day, the resistance of the right pad was alwaysgreater than that of the left. After preganglionic denervation of the left side, this consistent difference disappeared.

taneous determinations of resistance from a pad deprived of its postganglionic nerves and from a pad without preganglionic innervation. The skin resistances of one of these cats is presented in figure 3. After removal of the right sympathetic chain from above the stellate through T_7, the resistance of the right forepad was consistently greater than the resistance of the left forepad. When the left ventral spinal roots from T_1–T_9 were cut, the resistance of both pads increased enormously. As the animal recovered, the resistances decreased together until the twelfth day after the ventral root section. At that time the animal was used for another experiment.

In similarly operated cats which were allowed to live longer no significant differences between the forepads could be detected until about four weeks after the ventral root section, when the regenerating fibers of the cut ventral roots began to establish functional connections with the postganglionic neurons, and the resistance of the left forelimb began to fall (14). In four to five weeks the resistance of the left forepad had returned to its pre-operative level and reflex changes could be elicited by pinching the tail. The high resistance of the right forepad persisted until the death of the animal.

SUMMARY

Since our experiments and those of others (10, 11) have failed to show activity in isolated sympathetic ganglia, and since it has been demonstrated that preganglionic denervation based on section of the white rami is incomplete, we feel that the preponderance of evidence supports the idea that the sympathetic ganglia of the upper thoracic chain, when severed from the central nervous system, are incapable of independent activity.

REFERENCES

(1) Dogiel, A. S Arch. f. mikr. Anat. **52:** 44, 1898.
(2) Langley, J. N. Brain **26:** 1, 1903.
(3) Ranson, S. W. J. Comp. Neurol. **29:** 305, 1918.
(4) Schwartz, H. G. This Journal **109:** 593, 1934.
(5) Magoun, H. W., W. K. Hare and S. W. Ranson. Arch. Neurol. and Psychiat. **37:** 1237, 1937.
(6) Nonidez, J. F. and K. Hare. Unpublished observations.
(7) Zuckerman, S. Trans. Zoological Soc. London **23:** 315, 1938.
(8) Kuntz, A. The autonomic nervous system. Lea and Febiger, Philadelphia, 1934.
(9) Langley, J. N. J. Physiol. **20:** 55, 1896.
(10) Bolton, B., D. J. Williams and E. A. Carmichael. Brain **60:** 39, 1937.
(11) Bronk, D. W., S. S. Tower, D. Y. Solandt and M. G. Larrabee. This Journal **122:** 1, 1938.
(12) Govaerts, J. C. R. Soc. de Biol. **119:** 1181, 1935; **121:** 854, 1936.
(13) Tower, S. S. and C. P. Richter. Arch. Neurol. and Psychiat. **28:** 1149, 1932.
(14) Hinsey, J. C., R. A. Phillips and K. Hare. This Journal **126:** P534, 1939.

THE EFFECT OF STEROIDS OF THE ADRENAL CORTEX AND OVARY ON CAPILLARY PERMEABILITY

S. C. FREED AND E. LINDNER

From the Cardiovascular Department and Department of Medicine, Michael Reese Hospital[1]

Accepted for publication June 30, 1941

It has been demonstrated that the adrenal cortex has a significant relationship in the development of acute circulatory collapse. Some years ago it was shown that adrenalectomized animals were more easily put in a state of shock than normal ones by muscle trauma and other procedures, and that adrenal cortex extracts could ameliorate this lack of resistance to a considerable extent. More recently, it has been reported that desoxycorticosterone is effective in preventing the development of surgical shock and the peripheral circulatory failure of pneumonia, anesthesia, epinephrine and other agents. However, Swingle et al. (1) could not prevent the shock following intestinal stripping in adrenalectomized dogs by administering desoxycorticosterone acetate, confirming the results of Selye and Dosne (2) in intact rats, and of Weil et al. (3) in normal rabbits. Selye and Dosne have reported, however, that corticosterone administration was an effective measure for combating this type of shock.

A considerable amount of evidence has been presented which demonstrates that the adrenal cortex has an important function in regulating electrolyte and water metabolism of the body and it would appear that this property is involved in its relationship to peripheral circulatory failure. Darrow et al. (4) have postulated that the adrenal cortex regulates electrolyte metabolism by way of the kidney. On the other hand, Swingle et al. consider that the effect of the adrenal cortex in regulating body fluid is through the maintenance of the integrity of the peripheral vascular system.

The recent work of Menkin (5) is significant in this regard. He demonstrated that extracts of the adrenal cortex prevent or neutralize the effect of leukotaxin, a substance present in exudates which increases the permeability of skin capillaries. Menkin used adrenal cortex extracts which contained numerous steroids but he also indicated briefly that desoxycorticosterone had a similar effect. From these results it would appear that

[1] Aided by the A. D. Nast Fund for Cardiac Research and the Emanuel Friend Fund.

the rôle of the adrenal cortex in offsetting shock might be dependent on its ability to maintain capillary permeability.

Due to the fact, however, that the adrenal cortex extract and corticosterone behave somewhat differently from desoxycorticosterone in combating shock, it was decided to investigate the action of crystalline corticosterone and crystalline desoxycorticosterone acetate on capillary permeability. We have included in this study, the action of estrone, progesterone and stilbestrol since these possess some of the properties of cortical steroids in electrolyte metabolism.

METHODS AND RESULTS. Menkin's method of demonstrating changes in capillary permeability was followed with certain modifications. The various steroids used in our experiments were injected intracutaneously on the denuded abdomen of rabbits, alone and in combination with leukotaxin. Ten to twenty minutes later, 10 to 15 cc. of 1.5 per cent solution of trypan blue in physiological saline was injected intravenously and the concentration of dye at the sites of the intracutaneous injections was used as the index of change in capillary permeability. The crystalline steroids were water insoluble and since oil solutions were considered unsuitable for intracutaneous injection, these steroids were suspended in physiological saline or saline-gum acacia solution. The suspensions contained 1 to 5 mgm. of steroid per cubic centimeter and were fine enough to be drawn up into a 26 gauge needle.

Tests for the blank vehicles, i.e., saline and saline gum-acacia showed either no dye concentration or a blanched area. The following table indicates our results with adrenal cortex extract, corticosterone and desoxycorticosterone[2] on the permeability of the skin capillaries when injected by themselves and together with a solution containing leukotaxin. The local concentration of dye in the injected areas was graded by plus signs, i.e., "one plus" indicating a slight but definite concentration, "four plus," the maximum concentration. Doubtful concentration of dye is listed as "? plus."[3] Negative signs indicate similarly the degree of blanching, at the injected areas.

It can be seen from table 1 that the adrenal cortex extract neutralized the effect of leukotaxin in every instance and as a matter of fact, actually produced areas at the sites of injection which were paler than the untreated

[2] We are indebted to Dr. E. C. Kendall for crystalline corticosterone, Dr. V. Menkin for the preparation of leukotaxin, Dr. M. Gilbert (Schering Corp.) for crystalline desoxycorticosterone acetate and Dr. D. Klein (Wilson Laboratories) for adrenal cortex extract.

[3] The color due to dye concentration was unchanged following pressure with a glass slide on the affected skin, thereby ruling out the possibility that the color was the result of a dilatation of the local capillaries rather than an increase in capillary permeability.

skin. Corticosterone suspensions produced effects similar to adrenal cortex extract in preventing dye concentration. On the other hand, desoxycorticosterone was unable to neutralize the leukotaxin effect in every instance and actually induced a significant concentration of dye at times, when injected alone.

TABLE 1

*Effect of intracutaneous injections of adrenal cortex steroids on concentration of trypan blue**

	CORTICOSTERONE	CORTICOSTERONE WITH LEUKOTAXIN	DESOXYCORTICOSTERONE	DESOXYCORTICOSTERONE WITH LEUKOTAXIN	ADRENAL CORTEX EXTRACT	ADRENAL CORTEX EXTRACT WITH LEUKOTAXIN	LEUKOTAXIN
1	0	?+	0	++++	−−	0	+++
2	0	?+	0	+++	−	−	++
3	0	?+	+	+++	−−	−−	+++
4	0	0	++	+++	−−	−−	+
5	?+	0	+	+++	−−	−−	++
6	0		++		−		
7	0		0		−−		

* Desoxycorticosterone acetate was used. Each horizontal row represents results obtained in a rabbit during a single set of these experimental procedures.

TABLE 2

*Effect of intracutaneous injections of ovarian substances on concentration of trypan blue**

	ESTRONE	ESTRONE WITH LEUKOTAXIN	STILBESTROL	STILBESTROL WITH LEUKOTAXIN	PROGESTERONE	PROGESTERONE WITH LEUKOTAXIN	LEUKOTAXIN
1	+	+++	+	++	+++	++++	+++
2	0	+++	+	+++	+++	+++	0
3	+	++	0	++	++	+++	+++
4	0	+	+	+++	++	+++	0
5	+	++++	++	++	+++	+++	0
6	++	++	0	++	++	++	+
7	++	++++	+	+++	++	+++	0
8	0	++	++	+++	++	+++	+
9	+		++		++		

* Desoxycorticosterone acetate was used. Each horizontal row represents results obtained in a rabbit during a single set of these experimental procedures.

The following substances were similarly tested: estrone, stilbestrol and progesterone. Stilbestrol, though not a steroid, was included in order to determine whether its action on extra-gonadal tissues resembled that of estrone.

From table 2 it can be seen that these substances failed to neutralize leukotaxin, and at times induced a concentration of dye when injected

alone. Progesterone gave the most consistent effect in this regard. In several instances, both leukotaxin and the ovarian substances produced negligible dye concentrations when injected alone, but the combinations of leukotaxin with these substances resulted in a marked dye concentration.

The possibility was considered that the estrogens might liberate acetylcholine in the skin and thus produce their effects on the capillaries, since Reynolds (6) has indicated that estrogens increase the acetylcholine content of gonadal and extra-gonadal tissues. However, it was found that it required acetylcholine concentrations of 1 to 80 or even 1 to 20, to induce a moderate dye concentration. These amounts were often toxic or fatal to the test animals. Acetylcholine, therefore, appears to play no rôle in our results.

Discussion. It appears from our results that desoxycorticosterone, a substance which is concerned with salt and water metabolism, is unable to prevent an increase in capillary permeability under conditions where adrenal cortex extracts are effective. In addition, this substance may actually cause an increase in capillary permeability by itself. On the other hand, corticosterone which has relatively little effect on salt and water metabolism is quite capable of neutralizing the leukotaxin effect of increasing the permeability of skin capillaries. It is possible that the activity of adrenal cortex extracts in this respect is due to its content of corticosterone or similarly acting compounds.

From the above results it appears that the preventive action of desoxycorticosterone on shock following muscle trauma, anesthesia, epinephrine and intraperitoneal glucose is apparently not due to a maintenance of capillary permeability. It is more likely one of controlling blood volume through shifts in electrolyte content of body fluids. It is significant in this regard that Freed (7) was able to prevent the otherwise fatal shock of muscle trauma in adrenalectomized rats simply by administration of physiological saline. These results support the concept that shock following muscle trauma is primarily the result of hypohydremia. Of course, not all forms of shock are explained on this basis. Manipulation of the intestines produces a type of shock which cannot be prevented by desoxycorticosterone administration but can be prevented by adrenal cortex extracts or corticosterone (1–3). Thus, it would follow that in intestinal manipulation, changes in capillary permeability play a primary rôle.

In regard to the estrogen and progesterone effects, it appears that they simulate the action of desoxycorticosterone on the permeability of capillaries. It is noteworthy that the steroids able to maintain health and life in adrenalectomized animals, do not have identical effects on capillary permeability. It is likely, therefore, that the ability of steroids to maintain life following adrenalectomy is not necessarily related to their regulation of capillary permeability.

SUMMARY

1. Crystalline corticosterone, desoxycorticosterone and commercial adrenal cortex extract were tested for their effect on capillary permeability according to Menkin's "leukotaxin" method. Corticosterone as well as adrenal cortex extract prevented the action of leukotaxin in increasing the permeability of capillaries. Desoxycorticosterone did not do this but actually often produced a slight increase in capillary permeability by itself. These results may explain, in part, the different responses elicited by these preparations in protecting animals against the circulatory failure of various types of secondary shock.

2. Estrone, stilbestrol and progesterone were similarly tested. All of these substances not only failed to prevent the leukotaxin effect but produced an increase in capillary permeability when administered alone. It was demonstrated that these responses were not due to the local liberation of acetylcholine.

3. It is concluded that the ability of steroids to maintain the life of adrenalectomized animals is not necessarily related to their effects on capillary permeability.

We are indebted to Dr. L. N. Katz for his valuable suggestions in the course of this study.

REFERENCES

(1) Swingle, W. W., H. W. Hays, J. W. Remington, W. D. Collings and W. M. Parkins. This Journal **132:** 249, 1941.
(2) Selye, H. and C. Dosne. Lancet **239:** 70, 1940.
(3) Weil, P., C. B. Rose and J. S. L. Browne. Canad. M. A. J. **43:** 8, 1940.
(4) Darrow, D. C., H. C. Harrison and M. Taffel. J. Biol. Chem. **130:** 487, 1939.
(5) Menkin, V. This Journal **129:** 691, 1940.
(6) Reynolds, S. M. R. and F. I. Foster. This Journal **131:** 422, 1940.
(7) Freed, S. C. Proc. Soc. Exper. Biol. and Med. **30:** 677, 1933.

HEMOGLOBIN PRODUCTION INCREASES WITH SEVERITY OF ANEMIA[1]

F. S. ROBSCHEIT-ROBBINS AND G. H. WHIPPLE

From the Department of Pathology, The University of Rochester School of Medicine and Dentistry, Rochester, New York

Accepted for publication July 1, 1941

It is apparent from the experiments tabulated below that the hemoglobin output is stepped up as the anemia level of circulating hemoglobin sinks. This was not an unexpected reaction but we were surprised to find how closely the hemoglobin output was related to the intensity of the anemia stimulus in these standard dogs.

If we assume that the *stimulus* to hemoglobin production is *zero* in the normal dog which presents a circulating blood level of 21 grams hemoglobin per cent, we may safely say that the stimulus to hemoglobin production is *maximal* at an anemia level of about 6 grams hemoglobin per cent. This gives a maximal *anemia range* of 15 grams hemoglobin per cent in these dogs as used.

The *moderate anemia* level used in our experiments is 11 grams hemoglobin per cent or two-thirds of the anemia range of 15 grams hemoglobin per cent described above (21 grams − 11 grams = 10 grams hemoglobin per cent). The average values for hemoglobin production in moderate anemia are very close to two-thirds that found in the same standard dogs at the severe anemia level (table 1). These values obtain whether the dogs are tested with liver feeding or iron salts or liver extract given by mouth.

Stimuli which cause the body to produce blood proteins are not well understood—a magnificent understatement. Yet the adjustments in the normal dog are exquisite in their delicacy whether we consider the production of hemoglobin or fibrinogen or albumin of the blood. It is assumed generally that the stimulus of anoxemia is responsible for the production of new hemoglobin but there may be other stimuli related to this complex response (4).

EXPERIMENTAL OBSERVATIONS. The general experimental procedure used in these standard anemia experiments has been reviewed in detail elsewhere (5). In general every effort is directed to the maintenance of uniform conditions.

[1] We are indebted to Eli Lilly and Company for aid in conducting this work.

Moderate anemia. Severely anemic dogs (hemoglobin 6 grams per cent) are permitted to attain a hemoglobin level of 11 grams per cent by the addition of the liver extract "Lextron" to the basal bread ration. Thereafter the dogs are fed the basal bread ration alone for several weeks until

TABLE 1

Hemoglobin production increases with severity of anemia

Figures represent net Hb production per 2 weeks

DOG NO.	LIVER FED*		IRON BY MOUTH†		LIVER EXTRACT FED‡	
	Anemia level					
	Moderate Hb—11 grams per cent	Severe Hb—6 grams per cent	Moderate Hb—11 grams per cent	Severe Hb—6 grams per cent	Moderate Hb—11 grams per cent	Severe Hb—6 grams per cent
	grams	*grams*	*grams*	*grams*	*grams*	*grams*
34-3	59	96	47	49	56	98
	48	77	39	78		
		76		52		
34-145	74	102	59	58	77	109
	65	90	50	61		92
37-22	46	85	39	54	52	81
	50	92		50		87
		73		50		
				74		
				46		
35-2	62	102	44	87	73	122
	42	101	60	78		88
		74		52		62
		104		81		100
		99		84		
				97		
Average hemoglobin production	56	90	48	66	65	93

* Pig liver as fed averages 52 mgm. Fe per 300 grams daily.

† Iron by mouth = 40 mgm. Fe as amm. citrate.

‡ Liver extract as fed averages 270 mgm. Fe per day.

hemoglobin production is stabilized at a uniform base line level. The various diet factors are then tested under standard experimental conditions at the moderate anemia level. Other diet factors have been tested at this moderate anemia level but not in sufficient number to report. In general we may say that these experiments are in harmony with those tabulated below.

All dogs reported in table 1 were clinically normal, active, of uniform weight, with good appetite. In spite of this clinically normal state there is considerable variation in the response noted in repeat experiments on the same dog. Such variables are not rare in physiological experiments dealing with protein metabolism. In part they may be due to digestion factors, protein requirements other than new hemoglobin, and protein or iron stores in organs and tissues. Average values determined in several dogs from many repeat experiments we believe are significant.

Table 1 gives the *net hemoglobin production* over and above the control base line in each experiment. The four dogs used were well standardized and had been anemic under continuous observation for 4 to 6 years. During the greater part of this time the severe type of anemia had been maintained. The anemia level used in our published experiments (5) is 45 per cent of 13.8 grams hemoglobin or 6 grams hemoglobin per cent. This 6 grams hemoglobin per cent represents the optimum grade of severe anemia in these dogs. The dog can tolerate this degree of anemia without obvious clinical abnormality and loss of appetite. Below an anemia level of 5.5 grams hemoglobin per cent the dog may show clinical disturbance and the production of new hemoglobin may actually decrease. Obviously the anemia level should be kept as constant as possible by frequent bleedings with related blood volume determinations.

These dogs were raised in our own kennels from the strain used in all our anemia experiments and the normal hemoglobin level in these dogs is 20 to 21 grams hemoglobin per cent. They tolerate the customary regime very well and they continue a fairly uniform rate of hemoglobin production on various diets throughout a normal life cycle—often over 10 years continuously anemic.

The liver extract, 5.9 grams given daily ("Lextron"—Eli Lilly and Company) contains 270 mgm. Fe as fed. This extract (7) contains substances other than iron which have been shown to be potent for hemoglobin production in standardized anemic dogs. Cooked pig liver (300 grams fresh equivalent) as a standard test diet factor, is given daily for 2 weeks and all hemoglobin production is measured in grams per 2-week period.

It is to be noted that the responses to liver and liver extract are similar but the amount of contained iron very different. We have shown that factors other than iron in whole liver are responsible for some of the new formed hemoglobin. The group of food proteins represents one factor (2) but amino acids and related compounds (6, 1) may be at times responsible.

Discussion. When a *deficit* in circulating *hemoglobin* is produced (anemia) there is a relatively prompt response by the body (measured in days) with the production of new hemoglobin and red cells. It is obvious from these experiments (table 1) that the production of new hemoglobin

increases as the hemoglobin deficit (anemia) becomes more severe and there appears to be some parallelism between the hemoglobin output and the severity of the anemia. This is a relatively simple response as the new hemoglobin can be measured and hemoglobin cannot be put away in some large hidden reserve store.

When a *deficit* in circulating *plasma protein* is produced in the dog (hypoproteinemia) there is a complex response. There is a prompt (hours) inflow of needed plasma protein coming from reserve stores, also a more leisurely (days) appearance of new plasma protein coming from reserve stores and from food in the intestinal tract but this influx tends to bring the plasma protein levels back to normal. We believe that the plasma proteins and certain labile tissue proteins are in a fluid or dynamic equilibrium (3), which means that new plasma protein may move easily in or out of reserve stores held within tissue cells—a complex reaction which makes for uncertainty when one would measure the output of new plasma protein following plasma depletion. Nevertheless it is apparent that hypoproteinemia does stimulate new plasma protein accumulation within the circulating blood plasma.

When a *deficit* in *both hemoglobin and plasma protein* is produced simultaneously (4) we observe preference being given to hemoglobin production no matter what diet protein is utilized—a totally unexpected response which is worthy of much study. It seems probable that in some way the responses to anemia and hypoproteinemia are interrelated—that anoxemia is not the only stimulus concerned in the production of new hemoglobin.

SUMMARY

A severe anemia level of 6 grams hemoglobin per cent in dogs gives maximal stimulus for the production of new hemoglobin.

A moderate anemia level of 11 grams hemoglobin per cent under identical conditions gives a new hemoglobin production of approximately two-thirds of this maximum (table 1).

The term maximal *anemia range* is used to designate the difference between a normal blood of 21 grams hemoglobin per cent and a severe anemia of 6 grams hemoglobin per cent = 15 grams hemoglobin per cent. A moderate anemia (11 grams hemoglobin per cent) represents an anemia range of 10 grams (21 − 11 grams hemoglobin)—or two-thirds of the maximal anemia range.

The hemoglobin production therefore seems to run parallel to the degree of the anemia.

One stimulus to new hemoglobin production is believed to be anoxemia but there may well be other factors in this reaction.

REFERENCES

(1) György, P., F. S. Robscheit-Robbins and G. H. Whipple. This Journal **122:** 154, 1938.
(2) Hahn, P. F. and G. H. Whipple. J. Exper. Med. **69:** 315, 1939.
(3) Madden, S. C. and G. H. Whipple. Physiol. Rev. **20:** 192, 1940.
(4) Robscheit-Robbins, F. S., S. C. Madden, A. P. Rowe, A. P. Turner and G. H. Whipple. J. Exper. Med. **72:** 479, 1940.
(5) Whipple, G. H. and F. S. Robscheit-Robbins. This Journal **115:** 651, 1936.
(6) Whipple, G. H. and F. S. Robscheit-Robbins. J. Exper. Med. **71:** 569, 1940.
(7) Whipple, G. H., F. S. Robscheit-Robbins and G. B. Walden. Am. J. Med. Sci. **179:** 628, 1930.

ESTIMATION BY THE FOREIGN-GAS METHOD OF THE NET (SYSTEMIC) CARDIAC OUTPUT IN CONDITIONS WHERE THERE IS RE-CIRCULATION THROUGH THE LUNGS

ANCEL KEYS

From the Laboratory of Physiological Hygiene, University of Minnesota, Minneapolis, Minnesota

Accepted for publication July 1, 1941

In the course of studies on patients with patent ductus arteriosus (Keys, Violante and Shapiro, 1940; Shapiro and Keys, 1941) the estimation of the net (systemic) cardiac output became of interest. Eppinger, Burwell and Gross (1941) reflect the general belief that the foreign-gas methods cannot be applied to such conditions. We shall show that this belief is incorrect. The present paper is to present a general mathematical treatment for all conditions in which some fraction of the blood recirculates through the lungs and to give some results of the application of the relations which emerge from this theoretical analysis.

The considerations developed in this paper apply equally to nitrous oxide (Krogh and Lindhard, 1912), ethylene (Marshall and Grollman, 1928), ethyl iodide (Henderson and Haggard, 1925; Starr and Gamble, 1928), and to acetylene (Grollman, 1932). However, the acetylene method has been most widely used and is the method of choice in this laboratory, so the subsequent discussion will refer specifically to that method.

In the acetylene method Henry's Law applies, i.e., the amount of acetylene dissolved in the blood is proportional to the partial pressure of acetylene in the gaseous phase. Therefore, the amount of acetylene removed by the blood in unit time is directly proportional to the *effective* average partial pressure of acetylene in the lung-bag system and to the total blood flow through the lungs in unit time.

In the basic equation:

$$\text{(1)} \qquad \text{Cardiac output} = \frac{\text{oxygen consumption}}{\text{A.-V. oxygen difference}}.$$

The presence of a shunt of blood from the left to the right heart or equivalent does not affect the validity of the equation if we specify that the "cardiac output" refers to the net systemic output. Obviously, blood which has already been saturated with oxygen in the lungs and then re-enters the pulmonary circuit will not absorb more oxygen nor will it lose

any if the partial pressure of oxygen in the lungs is kept at 100 mm. or above. Such blood will not, in general, participate in the oxygen exchanges. With the foreign gas methods, however, the A.-V. oxygen difference is obtained from the concentrations of the foreign gas and hence this must be considered in detail.

Foreign-gas removal by the blood. The arterial-venous oxygen difference, which must be known in order to use equation (1) above, is equal to:

$$\text{(2)} \qquad \text{A.-V. diff.} = \frac{(\Delta O_2)(C_{av})}{(\Delta C)Q}, \text{ (Grollman, 1932)}$$

where ΔO_2 is the difference in oxygen concentration between the first and second samples (corrected for the volume change of the lung-bag system), C_{av} is the average concentration of acetylene in the lung-bag system during the period between samples, ΔC is the acetylene difference between the samples (corrected for volume change) and Q is a constant for the solubility factor for acetylene in the blood at any given barometric pressure and lung temperature. The cardiac output is, therefore:

$$\text{(3)} \qquad V = \frac{(BMR)(\Delta C)Q}{(\Delta O_2)(C_{av})}.$$

In equation (3), C_{av} is the true effective average partial pressure of acetylene during the period when the acetylene concentration change, ΔC, was brought about. Now if C_a and C_b represent the concentrations of acetylene in the lung bag system in the samples taken at times t_a and t_b respectively, then the true average value C_{av}, may be calculated from the integral of $\frac{-dC}{dt} = k$, or

$$\text{(4)} \qquad kt = \log_e\left(\frac{C_a}{C_b}\right). \quad \text{If } k' = \frac{k}{2.303}, \text{ then}$$

$$\text{(5)} \qquad k't = \log_{10}\left(\frac{C_a}{C_b}\right).$$

Equations (4) and (5) represent, of course, the theoretical equation for a reaction of the first order; experimental verification of this equation is available and will be discussed later. Accordingly, the average acetylene concentration in passing from C_a to C_b is

$$\text{(6)} \qquad C_{av} = \log^{-1}\left(\frac{\log C_a + \log C_b}{2}\right).$$

Now the *effective* acetylene concentration causing absorption of acetylene by the blood is the difference between the average concentration in the lung-bag system and that in the blood entering the lungs, or $\bar{C}_{av} =$

$C_{av} - C'_{av}$. If the blood entering the lungs is a mixture of ϕ parts of blood containing acetylene in equilibrium with C'_{av} concentration and $1 - \phi$ parts of blood containing no acetylene, then the *effective* average concentration will be $\bar{C}_{av} = C_{av} - \phi C'_{av}$ and the *total* cardiac output through the lungs will be

$$V' = \frac{(BMR)(\Delta C)Q}{(\Delta O_2)(C_{av} - \phi C'_{av})}. \tag{7}$$

The evaluation of C'_{av} can readily be made in terms of the shunt-lung circulation time y and the total time t between t_a and t_b when the lung-bag samples are taken. It can be shown that:

$$C'_{av} = C_{av}\left(\frac{C_a}{C_b}\right)^{y/t}. \tag{8}$$

Accordingly, equation (7) may be written:

$$V' = \frac{(BMR)(\Delta C)Q}{(\Delta O_2)\left(C_{av} - C_{av}\phi\left(\frac{C_a}{C_b}\right)^{y/t}\right)}, \tag{9}$$

and the true net output is

$$V_0 = V'(1 - \phi) = \frac{(BMR)(\Delta C)(1 - \phi)Q}{(\Delta O_2)(C_{av})\left(1 - \phi\left(\frac{C_a}{C_b}\right)^{y/t}\right)}. \tag{10}$$

Now suppose we have attempted to calculate this output by the simple equation (3). The value we should obtain would be, as per cent of the true net output, 100 times the right hand term of equation (3) divided by the right hand term of equation (9), or:

$$\text{Per cent true } V_0 = \frac{1 - \phi\left(\frac{C_a}{C_b}\right)^{y/t}}{1 - \phi} \times 100. \tag{11}$$

It will be noted from equation (10) that the true net (systemic) cardiac output will be underestimated if the simple equation (3) is used when there is a re-circulation because the ratio C_a/C_b must always be greater than 1.0 and hence the entire expression $\left(\frac{C_a}{C_b}\right)^{y/t}$ will also be greater than 1.0 no matter what may be the values of y and t. Furthermore, y/t will normally be only a fraction of 1.0 and $C_a - C_b$ is ordinarily small compared to C_a, i.e., C_a/C_b will be considerably less than 2.0, so the entire expression $\left(\frac{C_a}{C_b}\right)^{y/t}$ cannot be *much* greater than 1.0 and we may expect the error from the use of equation (3) to be small.

The magnitude of the error may be calculated for a sample case. The sample data given by Grollman (1932, p. 66) may be used. Here the corrected acetylene concentrations are $C_a = 10.76$, $C_t = 9.36$. We shall indicate later that the lung-circulation time, y, is of the order of 3 to 6 seconds. If we assume that $y = 5$ and that the time between samples is $t = 10$, we may calculate the effect of a "leak" or re-circulation of 40 per cent, that is, where 40 per cent of the blood ejected from the left ventricle passes through the ductus and makes a second transit of the lungs. In this case, then, the net cardiac output calculated by the ordinary equation (3) would be, as per cent of the true net output:

$$\text{Per cent } V_0 = \frac{1 - 0.4\left(\frac{10.76}{9.36}\right)^{5/10}}{1 - 0.4} \times 100 = 95.2,$$

and the error would be only -4.8 per cent.

When the ordinary calculation is made in cases with re-circulation the net output will be under-estimated by an amount dependent on the 3 factors, C_a/C_b, y/t and ϕ. We may consider the magnitudes of these factors individually.

The ratio C_a/C_b. In order to show what we may expect for values of C_a/C_b we have analyzed all the data in the last 2 notebooks of this laboratory, covering 89 consecutive acetylene experiments, 40 on patients with patent ductus arteriosus and 49 on normals and patients with heart disease not involving re-circulation. In this series C_a/C_b averaged 1.23, $\sigma = \pm 0.116$ in the patent ductus arteriosus patients and 1.25, $\sigma = \pm 0.085$ in the others. The extreme range was 1.07 to 1.44 with the exceptions of 2 experiments of dubious technical validity where the apparent ratio C_a/C_b was 1.47 and 1.57.[1] The person on whom the highest ratio, 1.57, was found, has since been studied on 3 occasions where we found the ratios 1.10, 1.18 and 1.28.

The ratio y/t. The value of t, the time between the gas samples, is ordinarily of the order of 10 to 15 seconds, but the proper timing has been the subject of some discussion (cf. e.g., Starr and Collins, 1933; Gladstone, 1935; Adams and Sandiford, 1941). It seems agreed that adequate mixing in the lung-bag system requires no more than about 6 to 8 seconds with proper breathing (op. cit.) and that has been our experience. Grollman (1932) reported that evidence of recirculation of systemic blood normally never appears before 23 seconds from the start of rebreathing and therefore the second gas sample can be taken at that time. Careful studies on a small number of persons in this laboratory indicate that an even longer

[1] These highest values, though questionable, were included in the averages cited above.

time, at least 26 seconds, may be used in subjects at rest. In normal subjects, then, t may be as long as 18 or 20 seconds.

Where there is a short-circuit re-circulation through the lungs, however, the first gas sample should not be taken before blood in equilibrium in the lungs begins its recirculation via the short circuit. In other words, mixing in the lungs should be completed at least y seconds before the first sample is taken. We must consider the lung-circuit time, y, before deciding on the proper duration of t.

The volume of blood in the lungs at any one time represents about 7 per cent of the total blood in the body (Spehl, 1883; Menicanti, 1894; Tigerstedt, 1903). From this we can calculate that, in rest, the lung-circuit time would normally be about 4 seconds. Starr and Collins (1933) found the lung-circuit time to be "about 5 seconds" in direct experiments. Where there is a shunt involving re-circulation the lung vessels may be somewhat engorged (Keys, Violante and Shapiro, 1940) but the velocity of the total blood flow is, of course, higher than normal so that the lung-circuit time in these cases may even be less than in normal persons.

From the foregoing, we conclude that the first gas sample should be taken at around 13 seconds and if the second sample is taken at 25 seconds the ratio y/t is about 0.3 and even when the second sample is taken at 23 seconds the ratio y/t should not exceed 0.5 in the absence of heart failure and pulmonary congestion.

The value of ϕ. The magnitude of the re-circulation, ϕ, in patent ductus arteriosus has been estimated in 3 cases by Eppinger, Burwell and Gross (1941) from oxygen analyses for blood taken at various sites and times during operation to close the ductus. In spite of the difficulties of proper sampling and the abnormal conditions—anesthesia and the chest open—the values calculated are of interest. In 2 cases it was suggested that about 50 per cent of the left ventricular output passed through the ductus; in the third case a value of 77 per cent was obtained. In the latter case the calculated left ventricular output seems impossibly high—25 liters per minute in a girl of 42 kgm. body weight with only "moderate cardiac enlargement." The general conclusion from these studies would be that ϕ may be around 0.5 in severe cases of patent ductus arteriosus.

We have studied 27 cases of patent ductus arteriosus with our simultaneous roentgenkymography and acetylene re-breathing methods (Keys et al., 1939, 1940). Repeated studies were made on many of these patients and the results were satisfactory in 22 of them. Even without correction for the re-circulation effect the comparison of the stroke-volume change of the heart with the acetylene removal from the lungs permits an approximate estimate of the value of ϕ. These (uncorrected) values for ϕ ranged up to 0.7 in several very severe cases and were of the order of 0.2 to 0.4 in the majority of the patients.

The range of values of ϕ in interventricular septal defects is entirely conjectural at present. In general, we may expect that the shunt of blood from left to right heart should be of the same order of magnitude as in patent ductus arteriosus.

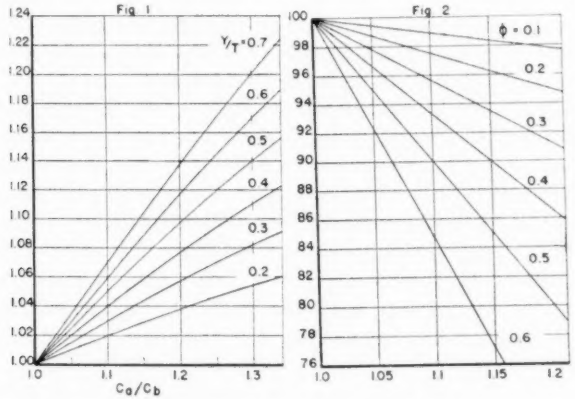


Fig. 1. Values, on the rectangular ordinate, for the expression

$$\left(\frac{C_a}{C_b}\right)^{y/t}$$

for various values of C_a/C_b and for y/t.

Fig. 2. Values for

$$100\left(\frac{1-\phi\left(\frac{C_a}{C_b}\right)^{y/t}}{1-\phi}\right),$$

on the rectangular ordinate, for various values of ϕ and for

$$\left(\frac{C_a}{C_b}\right)^{y/t}$$

(on the rectangular abscissa).

The evaluation of equation (11). In order to illustrate the magnitude fo the total error and to facilitate calculations for particular cases, we have prepared three-dimensional graphs. Figure 1 gives values for $\left(\frac{C_a}{C_b}\right)^{y/t}$ corresponding to values of $\left(\frac{C_a}{C_b}\right)$ up to 1.35 and y/t up to 0.7. Figure 2 gives the values for the uncorrected cardiac output as per cent of the true net output corresponding to recirculation up to 60 per cent and values of $\left(\frac{C_a}{C_b}\right)^{y/t}$ up to 1.22.

We have been able to use equation (11) to estimate satisfactorily the errors resulting from uncorrected application of the acetylene method in 22 patients with patent ductus arteriosus. The maximum error was −12 per cent in an experiment with patient R. T., where ϕ was about 0.6, C_a/C_b was 1.21 and y/t was about 0.4. In other words, the uncorrected calculation gave a result 12 per cent less than the true net systemic output of the heart. The average error was −3.36 per cent, $\sigma = \pm 3.19$ per cent for 41 separate experiments with the 22 patients.

The reaction order for foreign-gas absorption. We have already stated that the absorption of acetylene proceeds as a first order reaction. Theory demands this monomolecular reaction behavior and it is confirmed by the fact that a straight line is obtained when the logarithm of the acetylene content (or concentration corrected for volume change) in the lung-bag system is plotted against time (Gladstone, 1935). We have confirmed this result but such a test requires a very well trained subject and care in taking the gas samples at precisely the same phase of respiration. The test is much more simply made by plotting the acetylene removal against the oxygen removal.

If the blood flow and metabolism are constant, the removal of oxygen from the lung-bag system proceeds linearly with time so long as the oxygen partial pressure is not allowed to fall below the point where the hemoglobin is practically saturated with oxygen in the lungs. Accordingly the indication that the acetylene absorption conforms to an equation of the first order is obtained if the plot of the oxygen content in the lung-bag system against the logarithm of the acetylene content conforms to a straight line. For "contents" the concentrations, corrected for volume change may be used, of course.

Figure 3 reproduces the results of a typical experiment in which 9 successive gas samples were taken during rebreathing. Note that in such experiments with multiple sampling the volume change resulting from the withdrawal of the samples must be allowed for in calculating the contents or corrected concentrations of the lung-bag system. This point has been generally disregarded by investigators using multiple sample procedures.

It is clear that the acetylene removal conforms closely to the expectation of a first order equation and that the rate of acetylene removal is constantly proportional to the acetylene concentration from the time equilibrium is established (sample 3) for 6 samples, a period of 27.7 seconds in this case. Similar results have been obtained with all other subjects tested, but the period of constant removal rate is usually somewhat shorter, averaging about 22 seconds in rest.

Experiments like that depicted in figure 3 verify equations (4) and (5) above and show that the final gas sample can be taken later than is currently believed possible. The reason for the long "safe" period for

sampling is undoubtedly that the first blood which carries acetylene in the systemic circulation loses most of this acetylene in equilibrating with the tissues before it returns to the heart (cf. Starr and Collins, 1933).

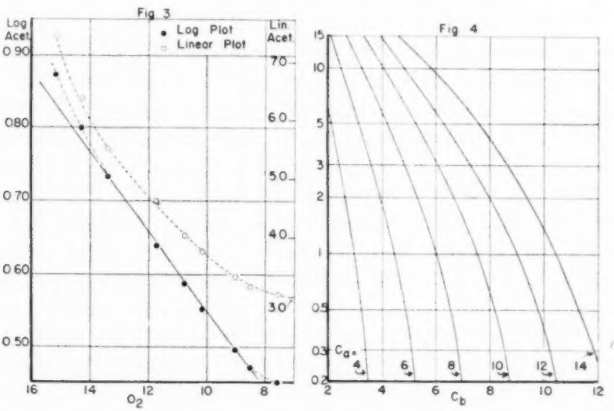


Fig. 3. Results of a typical acetylene re-breathing experiment in which 9 successive gas samples were taken. The acetylene and oxygen values in these samples have been corrected for the changes in the volume lung-bag system resulting from gas absorption in the lungs and from the withdrawal of the gas samples. Acetylene values on the ordinates, logarithmic scale on the left, linear on the right.

Fig. 4. Values for $-E$ in equation (14) on page 276, for various values of C_a and C_b. E is the error, in per cent of the true value, resulting from the use of the ordinary Grollman calculation for the average acetylene concentration in the lung-bag system between samples.

Average acetylene concentration. It is obvious that the average acetylene concentration should be calculated according to equation (6) above. Conversely, the ordinary Grollman calculation:

$$C''_{av} = \frac{C_a + C_b}{2} \neq C_{av}. \tag{12}$$

It is important, therefore, to discover the magnitude of the error resulting from the use of equation (12) rather than equation (6). It is readily shown that the error resulting if the Grollman equation (12) is used would be, as per cent of the true output:

$$E = 100\left(1 - \frac{V''}{V_0}\right) = 100\left(1 - \frac{1/2(C_a + C_b)}{\log^{-1}1/2(\log C_a + \log C_b)}\right). \tag{13}$$

It will be noted that E must always be negative since the ratio of C''_{av} to C_{av} will always exceed 1.0; i.e., the ordinary Grollman calculation will

always over-estimate the true average acetylene concentration and accordingly under-estimate the cardiac output. For this reason and to expedite calculation, equation (13) is more conveniently used in the form:

$$(14)\quad -E = \log^{-1}\left(2\log\tfrac{1}{2}(C_a + C_b) - \tfrac{1}{2}(\log C_a + \log C_b)\right) - 100.$$

Application of equations (13) or (14) to specific cases shows that the error is generally small and frequently entirely negligible. For example, E in the sample case from Grollman (1932, p. 66) is only -0.18 per cent. In some instances, however, it may be of consequence. Evaluation may be made from figure 4 in which we have plotted values for E for all values of C_a and C_b likely to be encountered in combination.

Oxygen exchanges. The negligible rôle of oxygen exchanges in cases of recirculation has already been mentioned. This is not true if the oxygen partial pressure falls below the level at which the hemoglobin of the blood is effectively saturated in the lungs. At body temperature and a partial pressure of $CO_2 = 40$ to 50 mm., human blood is about 96 per cent saturated with oxygen at 80 mm. Hg and 94 per cent at 70 mm. This means that, if the barometric pressure is 760 mm. the oxygen concentration in the lung-bag system would have to fall to around 10 or 11 per cent to produce a slight reduction in the rate at which oxygen is removed by the blood hemoglobin. It should be remembered that the oxygen gradient between alveolar air and arterial blood is only 1 or 2 mm. when the oxygen partial pressure in the lungs is of the order of 70 or 80 mm. (cf. e.g., Keys, 1938, p. 610).

The effect of changing partial pressure of oxygen in the lung-bag system on the oxygen removed in simple physical solution in the water of the blood is, of course, directly proportional to the oxygen partial pressure in the blood. If the arterial oxygen saturation falls as low as 90 per cent at the time of the second gas sample this would mean that the physically dissolved oxygen would only be about 0.04 volume per cent less than at the start. Since the total arterial-venous oxygen difference is of the order of 6.0 volumes per cent the maximum error from changing oxygen in physical solution is only about 0.7 per cent.

Coronary circulation. If the re-circulation involved tissues which themselves use up a large amount of oxygen from the blood these arguments would not apply. Such might be argued for the special case of the coronary circulation where the situation is complicated for both oxygen and acetylene or other foreign gas. Hamilton, Spradlin and Saam (1932) and Gladstone (1935) have suggested that the return of blood from the nearest circuits, especially the coronaries, constitutes a re-circulation that begins so quickly after the start of re-breathing that the whole experimental period should be made very short—less than 15 seconds.

A detailed analysis of the coronary re-circulation effect would be too

lengthy for inclusion here but we may state that the oxygen usage by the heart itself is a part of the total metabolism and so its effect on the oxygen exchanges need not be specially considered. Blood going through this system would, however, return rapidly to the lungs and would tend to alter the acetylene exchanges on re-entry. Two points may be noted.

In the first place, the heart itself would absorb a considerable part of the acetylene contained in the blood making its first circuit through it and would tend to equilibrate with it with further portions of blood. The solubility of acetylene in muscle is not greatly inferior to that in blood and may even exceed it if much fat is present. In the second place, the coronary blood flow is only of the order of 10 per cent of the total cardiac output. Equations (10) and (11) apply and ϕ is about 0.1. It does not seem possible for the coronary flow to introduce any considerable or even appreciable error. Even if y/t should be as large as 0.7 or 0.8 the resulting error is only of the order of 2 per cent.

The cardiac index in patent ductus arteriosus. In all we have made 41 satisfactory measurements of the net cardiac output under basal resting conditions in 22 patients with simple patent ductus arteriosus. None of these patients was in failure. By "satisfactory" is meant that all technical details were unexceptional, duplicate analyses agreed and adequate bases were available to estimate the approximate correction for the re-circulation effect. This last averaged 3.36 per cent, with a maximum of 12 per cent in one patient. The experiments were all made in a quiet room at 78°F. Body surface was computed from the height and weight by the charts of DuBois (1936).

The cardiac index, liters of blood per square meter of body surface per minute, averaged 2.45, $\sigma = \pm 0.520$, minimum 1.44, maximum 3.77, in this series. The average minimum for any one patient was 1.55, and the average maximum was 3.28. In 4 patients the cardiac index averaged less than 2.0. The range of these values is greater than and the average is slightly higher than for the large series of normal subjects studied in this laboratory. It is notable that the higher values tended to occur in patients with elevated metabolism.

The patients in this group averaged 15.7 years in age, ranging from 5 to 35 years old. We are unaware of any acceptable standards for cardiac index on a group of normals of comparable age but all indications are that the basal cardiac index is not much different in a group of this age composition from that found in young adults. The average cardiac index for normal young adults in this laboratory is about 2.4 under the same conditions used with the patients.

It is clear that the average cardiac index in our series of patients with patent ductus arteriosus is either normal or close to it. In 4 patients (16.7 per cent) the index is definitely subnormal, but in 5 patients (20.8

per cent) the index seems to be above the normal expectation. This is in full agreement with our results with patients with compensated heart disease of other types.

Discussion. The analyses and arguments advanced here apply only to conditions where the timing of the gas samples is reasonably correct. The first sample should not be withdrawn before blood from the short circuit, containing acetylene in equilibrium with the mixed gas, has made a re-entry into the lungs. The requirement that the second sample be withdrawn before the rate of acetylene absorption in the lungs changes is not likely to be unfulfilled in rest, as pointed out above.

We may inquire what would be the result if the first sample is withdrawn too early, say after mixing has taken place but before blood in equilibrium with the mixed gas has been able to re-enter the lungs. For example, suppose mixing is complete in 8 seconds, the lung-circuit time is 4 seconds and the first sample is taken at 10 seconds and the second at 22 seconds. The worst imaginable condition would be where for 2 seconds re-circulated blood containing *no* acetylene would be entering the lungs where it would, of course, absorb acetylene just like the true venous blood. This would be the equivalent of a relative blood flow of $1 + \phi$ instead of 1 for $\frac{1}{6}$ the period of measurement. If ϕ were 0.5 the result would be that the net circulation would be over-estimated by about 10 per cent.

Actually, during the initial 2 seconds of the period between samples the re-circulated blood entering the lungs would have far more than zero acetylene concentration. The proper expectation may be gauged from measurements of the rate of change of acetylene concentration in the lung-bag system during 3 or 4 seconds before mixing is complete. Experiments on this point indicate that in this period the alveolar air is less concentrated in acetylene than the bag gas but that if complete mixing takes 8 seconds the concentration in the alveolar air is not less than 80 per cent of the concentration in the bag gas at 6 seconds. Accordingly the error in the case cited would only be an overestimate of the order of 1 per cent.

It is obvious that the danger of error of the type just discussed is slight. Here, as elsewhere in this paper, the conclusions with regard to timing refer only to resting conditions and do not apply to exercising subjects.

Many arguments and suggestions have been made in the literature as to large and difficultly controlled sources of error in the foreign-gas methods for the estimation of the cardiac output. We may divide these into 2 groups, those having to do with standardization of physiological conditions and excitement, and those having to do with re-circulation, mixing, analysis and computation. The present paper has to do with the latter group of potential sources of error. It seems that these are nothing like so serious as is frequently supposed and, in general, they may be almost completely

eliminated if attention is paid to the lessons of considerations like those developed here.

On the other hand we are convinced that the difficulty of physiological standardization is generally underestimated and this applies not only to the foreign-gas methods but to all other methods for circulation measurement. In our experience it is more difficult to attain reproducible cardiac output standardization than metabolic standardization and the environmental and psychological conditions must be more rigorously controlled than for basal metabolism measurements. We deplore, therefore, the fact that this point is often neglected and this to an increasing extent as estimations of cardiac output are extended to a larger number of laboratories and hospitals.

SUMMARY

Some fundamental kinetics have been analyzed for the gas absorption by the blood in the foreign-gas methods for estimation of the cardiac output in man. The discussion applies specifically to the acetylene method but the conclusions apply to the other foreign gases.

It is shown that the absorption proceeds according to an equation of the first order and the mathematical analysis is developed accordingly.

The condition where re-circulation occurs in the lungs is analyzed in detail and equations are derived for the proper computation of the true systemic circulation in such cases. The variables involved are the concentrations of the foreign gas in the gas samples from the lung-bag system, the fraction of blood re-circulated, the short-circuit time, and the total time between samples. Graphs for computations with these variables are presented.

It is shown that re-circulation through the lungs in conditions such as in patent ductus arteriosus or in inter-ventricular septal defects does not necessarily introduce a serious error and that this error may be estimated.

It is shown that re-circulation of blood from the coronary system cannot introduce an appreciable error.

The error resulting from the common assumption that the absorption of the foreign-gas is linear with time is discussed and shown to be ordinarily small. Means are provided to estimate this error by equations and a graph.

Timing of the gas sampling is discussed. It is shown that the second sample may be taken later than is frequently believed possible. When there is re-circulation the first sample should be delayed 4 or 5 seconds but it is shown that 2 or 3 seconds are not critical.

Results of 41 studies on 22 patients with patent ductus arteriosus are presented in summary form. The average cardiac index is normal or close to it in this group.

It is indicated that many criticisms of the foreign-gas methods are in-

valid because they are based on quantitative misconceptions. On the other hand the importance and difficulty of physiological standardization in cardiac output measurements are frequently under-estimated.

Acknowledgment. It is a pleasure to record my obligations to Dr. M. J. Shapiro, who supplied most of the patent ductus patients, and to Drs. C. A. McKinley and Arild Hanson, who also referred patients, and especially to Dr. Antonio Violante and Miss Angie Mae Sturgeon who assisted in the experiments.

REFERENCES

(1) Adams, W. and I. Sandiford. J. Clin. Investigation **20:** 87, 1941.
(2) DuBois, E. F. Basal metabolism in health and disease. Lea and Febiger, Philadelphia, 1936.
(3) Eppinger, E. C., C. S. Burwell and R. E. Gross. J. Clin. Investigation **20:** 127, 1941.
(4) Gladstone, S. A. Cardiac output and arterial hypertension. New York, privately printed, 1935.
(5) Grollman, A. The cardiac output of man in health and disease. Springfield, Illinois, C. C. Thomas, 1932.
(6) Hamilton, W. F., M. C. Spradlin and H. G. Saam, Jr. This Journal **100:** 587, 1932.
(7) Keys, A. Ergebn. d. inn. Med. und Kinderheilkunde **54:** 585, 1938.
(8) Keys, A. and H. L. Friedell. This Journal **126:** 741, 1939.
(9) Keys, A., H. L. Friedell, L. H. Garland, M. F. Madrazo and L. G. Rigler. Am. J. Roentgen. Rad. Therap. **44:** 805, 1940.
(10) Keys, A., A. Violante and M. J. Shapiro. Arch. Lat. Amer. Cardiol. y Hematol. **10:** 237, 1940.
(11) Krogh, A. and J. Lindhard. Skand. Arch. Physiol. **27:** 100, 1912.
(12) Marshall, E. K., Jr. and A. Grollman. This Journal **86:** 117, 1928.
(13) Menicanti, G. Ztschr. Biol. **30:** 439, 1894.
(14) Shapiro, M. J. and A. Keys. Internat. Clinics, in press.
(15) Spehl, P. De la répartition du sang circulant dans l'economie. Brussels, 1883.
(16) Starr, I., Jr. and C. J. Gamble. This Journal **87:** 450, 1928.
(17) Starr, I., Jr. and L. H. Collins, Jr. This Journal **104:** 650, 1933.
(18) Tigerstedt, R. Skand. Arch. Physiol. **14:** 259, 1903.

TOLERANCE OF THE NEWBORN TO ANOXIA[1]

J. F. FAZEKAS, F. A. D. ALEXANDER AND H. E. HIMWICH

From the Departments of Physiology and Pharmacology and Anesthesia, Albany Medical College, Union University, Albany, New York

Accepted for publication July 1, 1941

From many points of view it is important to make a comparative study of the tolerance of the newborn and adult animal to oxygen lack whether produced by partial or complete replacement of oxygen of the inspired air by nitrogen, or by respiration of pure helium, nitrous oxide, cyclopropane, or carbon dioxide. These studies were not limited to the newborn but also include animals at various ages postpartum as well as fetuses in utero. Kabat (1) has already demonstrated the comparatively longer survival time of the newborn during asphyxia.

METHOD. Adults and infants of the following species were studied: rat, rabbit, cat, dog and guinea pig. Each animal was placed in a jar so arranged that its contents could be rapidly replaced by any desired gas. In some experiments the temperature of the jar and that of the animal within were recorded. The criterion used to indicate survival was the persistence of respiratory efforts, the end point being the time when these movements could no longer be evoked. The effect of the injection of 5 mgm. of sodium cyanide was studied both in young and adult animals breathing air. Estimations of the blood sugar level, by the Hagedorn and Jensen method (2), were made on samples of blood collected from rats and puppies before and during anoxia. The blood obtained from the puppies was also analyzed for oxygen contents by the method of Van Slyke and Neill (3). Electrocardiograms were recorded from these puppies. In order to estimate the influence of the brain on the length of the survival period, cerebral tissues were excised from rats of various ages and the oxygen consumption was determined in the Warburg apparatus. Pregnant animals respired mixtures of 5 per cent oxygen in nitrous oxide until exitus. The fetuses were then removed by Cesarean section and their behavior observed. Some of the pregnant animals were given lethal doses of sodium cyanide. After the mother succumbed, the fetuses were studied.

RESULTS. The adult rat exposed to an atmosphere of pure nitrogen undergoes a short period of excitement, becomes comatose, and succumbs after approximately 1.5 minutes. Figure 1 demonstrates that rats 1 day

[1] Aided by a grant from the Child Neurology Research (Friedsam Foundation).

of age survive for about 50 minutes at an environmental temperature of approximately 24°C. The points on the curve represent averages of numerous observations. As the rat progresses in age his tolerance to anoxia decreases and at approximately 17 days of age the sensitivity is like that of the adult. The oxygen consumption of minced cerebral tissue of infant rats of various ages reveals the following. From 1 to 10 days there are no significant changes. The cerebral metabolic rate, however, is well below that of the adult (4, 5). After 10 days of age there is a rapid increase of the oxygen uptake of the infant rat brain.

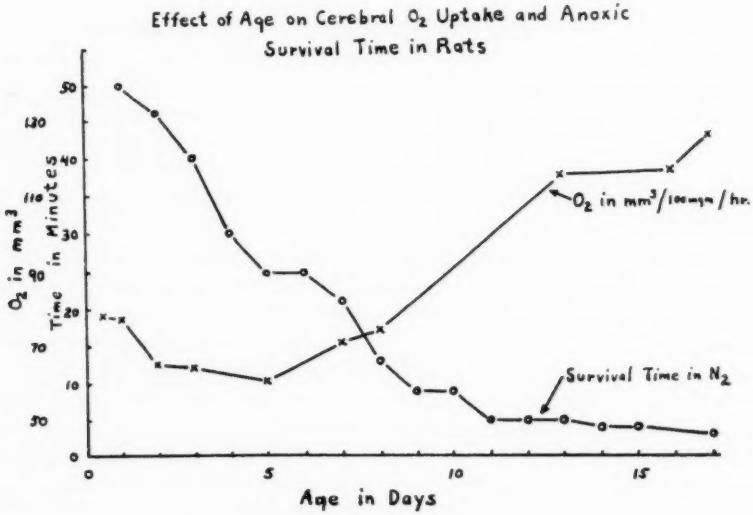


Fig. 1

The temperature of the newborn which approximates that of his environment has an important influence on the survival period. In a raised environmental temperature the survival period is decreased. At 1, 2, 4, 6 and 7 days of age the average survival periods were reduced and varied from 21 to 8 minutes as a result the environmental temperature being increased from 24 to 34°C. At either temperature the newborn far outlasts the adult.

Studies made on 26 newborn dogs reveal a survival period in nitrogen which may be extended to 43 minutes for puppies 1 day of age who are members of a vigorous litter. This period becomes shorter during the first week of life and in 1 observation of an animal 1 month of age, the time was reduced to 13 minutes. Analysis of repeated samples of arterial blood discloses that it contains practically no oxygen within the error of the

method after several minutes of nitrogen inhalation. The blood sugar demonstrates the asphyxial rise. Table 1 presents the survival period, blood glucose, and oxygen contents of some of the puppies studied. Among the newborn of other species it has been observed that on the average 5 kittens respired from 20 to 30 minutes, 5 rabbits for about 17 minutes, and 6 guinea pigs only 7 minutes in an atmosphere of nitrogen.

The same differential effect produced by nitrogen can also be demonstrated by the use of sodium cyanide. Concentrations which are lethal

TABLE 1

Survival periods of infant dogs respiring nitrogen

AGE	PERIOD IN NITROGEN	CONDITION AT TIME OF REMOVAL*	TIME OF BLOOD COLLECTION	OXYGEN CONTENTS ART. BLOOD	BLOOD SUGAR
days	*minutes*		*minutes*	*volume per cent*	*mgm. per cent*
1	16	D	14	0.23	
1	20	A†			
1	20	D	8	0.71	209
1	22	A	22	0.02	
1	29	A	13	0.02	
1	33	A	33	0.00	
1	39	A	39	0.00	
1	43	D	43	0.60	
2	24	D	4	0.36	
2	25	A	25	0.72	
4	22	A			
5	8	A			
6	28	D	5	0.04	104
			11	0.12	354
			16	0.05	336
			21	0.05	394
7	17	A	17	0.07	
13	10	A	3	0.71	131
			9		343
30	13	D			

* D = dead; A = alive.

† After 2 months animal in good condition.

to adult rats in 5 minutes permit infants to make respiratory efforts for an average of 61 minutes. It is interesting that the replacement of oxygen with nitrous oxide presents results exactly comparable with those of nitrogen. Rats 1 day of age survive for approximately 48 minutes. This duration decreases with age. At 1, 2, 3, 6 and 8 days the period of tolerance averages 52, 43, 36, 21 and 13 minutes respectively. Experiments on newborn rats in helium disclose results similar to those observed with nitrogen and nitrous oxide. Rats and dogs subjected to 5 per cent oxygen in nitrous oxide far outlast those respiring pure nitrous oxide. The adult

rat lives for 14 to 20 minutes and the infant usually at least 12 hours. Two adult dogs survived for about 15 minutes while 4 infants in the same mixture lived from 50 to 200 minutes. Baby rats do not tolerate carbon dioxide and cyclopropane as well as nitrous oxide but far outlive adults exposed to the same mixtures. The survival times with pure carbon dioxide at 1, 2, 3, 4, 5, and 10 and 12 days of age are 23, 28, 13, 13, 7 and 5 minutes respectively (25 observations). Twenty-four 1 day old rats lived in cyclopropane from 13 to 35 minutes. Sixteen newborn rats submerged in water at 37°C. continue to make respiratory movements for long periods. They survive at least 40 minutes, recover, and apparently develop normally. When the water contained india ink, granules of that substance

TABLE 2

Survival periods of newborns of different species subjected to various procedures

NUMBER OF OBSERVATIONS	AVERAGE SURVIVAL PERIOD	AGENT	SPECIES
	minutes		
150	50	Nitrogen	Rat
5	25	Nitrogen	Cat
18	23	Nitrogen	Dog
5	17	Nitrogen	Rabbit
6	7	Nitrogen	Guinea pig
17	20	Nitrogen (32-35°C.)	Rat
15	48	Nitrous oxide	Rat
11	26	Carbon dioxide	Rat
24	24	Cyclopropane	Rat
16	40	Submersion in water	Rat
9	61	Sodium cyanide	Rat
6	52	Helium	Rat

could be detected in the lungs of those animals that were sacrificed. The above results on the newborns are summarized in table 2.

Five pregnant animals, 1 cat and 4 rats were subjected to a mixture of 5 per cent oxygen in nitrous oxide until exitus. The adult cat succumbed after 10 minutes but all her 4 fetuses respired spontaneously on removal and survived until sacrificed. Similar results were observed on the rats. Despite the anoxic death of the mother and the fact that the fetuses were permitted to remain in utero 5 minutes thereafter some of them were successfully raised by foster mothers. Fetuses breathing spontaneously were delivered from pregnant rats given a lethal concentration of sodium cyanide. These infants survived for varying periods.

Four to 5 day old infant dogs which breathed pure nitrogen for 7 to 10 minutes showed the following electrocardiogram changes: first sinus arrhythmia, then slowing of rate succeeded by disappearance of the P wave within the first two minutes. Vagal dominance is probably indicated

during this period. Ventricular escape follows with nodal beats, idioventricular rhythm at a rate of 35 to 50 per minute. Then, possibly due to a diminution of the vagospasm, the P wave returns and after 5 minutes of anoxia, the rate becomes more regular and faster than after the first 2 or 3 minutes. If the anoxia is still further prolonged, heart action gradually weakens and cardiac standstill may be produced.

In one puppy there was a slight elevation of the ST segment. Ectopic beats, auricular fibrillation and flutter were seen in another puppy after 8 minutes. In different puppies SA node block, prolonged AV interval, occasional AV block, and bundle branch block were seen. Generally, with cessation of the anoxia the original rates returned with only slight irregularities. The changes in the electrocardiogram are essentially similar to those observed in adults but require more profound anoxia for development. It is significant that the heart continues to beat long after it is possible to evoke respiratory effects.

Discussion. From these results it may be concluded that under a wide variety of conditions the newborn exhibit a greater tolerance to anoxia than the mature of the same species. Resistance, however, does vary among species and seems to depend upon the degree of physiological maturity at the time of birth. For example, the newborn rat, which survives for approximately 1 hour, is without hair or teeth, has unopened eyes, is totally dependent on its mother and acts like a bulbo-spinal animal. The newborn guinea pig, on the other hand, which lives in an atmosphere of pure nitrogen for only 7 minutes is a comparatively mature animal exhibiting coördinated locomotion, righting reflexes, temperature regulation and therefore a functioning cephalad portion of the brain stem. In all species there is a loss of tolerance at a rate which seems to depend upon post natal development. For example, the rapidly developing rat at 18 days of age exhibits the diminished adult tolerance while the more slowly maturing dog is more resistant than the adult at 30 days of age. Kabat (1), who studied the resistance of the canine brain to cerebral anemia, observed that the tolerance of the young dog gradually decreases until 100 days of age, at which time it is indistinguishable from the adult. At this point it is interesting to compare the ages at which the oxygen consumption of excised brain of rat and dog attain the higher values of the mature animal. In the rat at 24 days of age the oxygen consumption of the brain is like that of the adult (4) while the higher oxygen uptake of the mature dog is developed in the infant when it attains the age of 35 to 50 days (6).

Since sodium cyanide inhibits practically all tissue respiration by inactivating the heavy metal carrying respiratory pigments, it might have been expected that the response to sodium cyanide would be similar to that with nitrogen. Nitrogen acts by displacing oxygen while cyanide prevents the utilization of that gas.

Despite a specific narcotic action of nitrous oxide the survival period of

the infant rat in that gas was similar to nitrogen. Perhaps the lack of the development of the most cephalad portion of the neuraxis, the part of the brain where nitrous oxide may exert its primary narcotic effect, is the reason for the lack of specificity. At a pressure of 1 atmosphere the action of nitrogen and helium are also similar on newborn and adult rats. These results are unlike those obtained with pressures greater than 1 atmosphere where nitrogen has a specific narcotic effect on the central nervous system (7).

The tolerance is not the same to all the gases studied. With carbon dioxide the survival period of the infant, though shorter than with nitrogen, was nevertheless still much longer than that of the adult in carbon dioxide. Among the factors which may explain this diminished survival time of the infant are the acidity of carbon dioxide and its narcotic action. Cyclopropane similarly shortens survival and exerts profound depression of respiration. The newborn rat in cyclopropane exhibits prolonged periods of apnea.

It is worthy of note that despite respiratory movements by infant rats, in some cases for 40 minutes, when submerged under water no immediate untoward effects are observed. From these experiments it may be seen that at least in the newborn rat respiratory movements in water for a period of 40 minutes have no deleterious action on the continued function of the respiratory mechanisms.

Since the newborn is more tolerant to anoxia than is its mother, it would be surprising if this same phenomenon did not hold antepartum. In our experiments with pregnant rats and cats, which were subjected to 5 per cent oxygen in nitrous oxide, a concentration lethal to the mother in approximately 10 minutes left the fetus capable of spontaneous respiration as observed after delivery by Cesarean section. These infants in many instances were making respiratory movements in utero. An accessory factor making for the survival of the infant during hypoxia may be the character of fetal hemoglobin which takes up relatively large amounts of oxygen even at low pressures of that gas.

The long period of tolerance to nitrogen of infant dog and rat facilitates the observation of respiratory changes in response to anoxia. They disclose a resolution of the phylogenetic development of the respiratory mechanisms. First apneusis develops with a long inspiratory phase indicating a depression of the pneumotaxic centers in the pons and changes of vagal activity (8). Next the apneusis becomes biphasic in character, as the long inspiration is divided in two by a short expiratory effort. Apneusis is succeeded by gasping respiration as only the lower medullary centers are left functioning. Finally after spontaneous respiration ceases there is a period during which respiratory gasps may be evoked by peripheral stimulation. If at any time the animals are permitted to respire air a recapitula-

tion of the respiratory changes is exhibited in the reverse order to that which is produced by anoxia.

In a consideration of the factors which permit the prolonged survival of the infant, the observation that increase of the temperature shortens survival time indicates the importance of metabolic rate. Thus the poikilothermia exhibited by the newborn rat may be considered a protective mechanism. The studies of the cerebral metabolism indicate that the low rate in the newborn may facilitate survival. For example, in the rat after the 10th day postpartum when the rate of cerebral metabolism begins to rise rapidly the survival period approaches the short adult time. Brain metabolic rate is not the only factor for it does not undergo significant changes during the first 9 days of life, a time during which tolerance to anoxia decreases rapidly. Since the puppies in nitrogen survive despite anoxia, it is obvious that there must be anaerobic sources of energy. These anaerobic mechanisms will be discussed in the subsequent paper.

SUMMARY AND CONCLUSIONS

A study is made of the tolerance to anoxia of the adult and infant of various species, rat, dog, cat, rabbit and guinea pig. Anoxia was produced by the respiration of nitrogen, nitrous oxide, helium, carbon dioxide, and cyclopropane and submersion in water. The newborn exhibit an extraordinary tolerance in comparison with the mature animals of the same species. The period of tolerance, however, is not the same in the various species studied being longest in the physiologically immature newborn rat and shortest in the comparatively mature guinea pig. Blood studies reveal that newborn dogs survive despite the fact that their arterial blood contains practically no oxygen after the first few minutes of anoxia. Among the factors permitting survival of the newborn rat and puppy is poikilothermia, a fall of temperature diminishing the metabolic demands. Another factor is the lower cerebral metabolic rate demonstrated in the rat and dog. Fetuses delivered from mothers who had succumbed to anoxia produced by inhalation of 5 per cent oxygen in nitrous oxide or the injection of cyanide respired spontaneously on delivery and some were raised by foster mothers.

REFERENCES

(1) Kabat, H. This Journal **130:** 588, 1940.
(2) Hagedorn, H. C. and B. N. Jensen. Biochem. Ztschr. **135:** 46, 1923.
(3) Van Slyke, D. D. and J. M. Neill. J. Biol. Chem. **61:** 523, 1924.
(4) Himwich, H. E., Z. Baker and J. F. Fazekas. This Journal **125:** 601, 1939.
(5) Himwich, H. E., P. Sykowski and J. F. Fazekas. This Journal **132:** 293, 1941.
(6) Himwich, H. E. and J. F. Fazekas. This Journal **132:** 454, 1941.
(7) Behnke, A. R. Ann. Int. Med. **13:** 2217, 1940.
(8) Pitts, R. F., H. W. Magoun and S. W. Ranson. This Journal **126:** 689, 1939.

THE EFFECT OF ANOXIA ON THE ABSORPTION OF GLUCOSE AND OF GLYCINE FROM THE SMALL INTESTINE[1]

DAVID W. NORTHUP AND EDWARD J. VAN LIERE

From the Department of Physiology, West Virginia University Medical School, Morgantown, West Virginia

Accepted for publication July 11, 1941

Cori (1925) has shown that the absorption of glucose (and galactose) is considerably more rapid than that of other monosaccharides such as mannose and xylose. He concluded that an active process is involved in the absorption of the former. Willbrandt and Laszt (1933) found that monoiodoacetate reduced the rate of absorption of glucose to that of mannose and xylose, indicating that the process involved phosphorylation of the glucose molecule. This work has been amply confirmed (Verzar and McDougall, 1936). Furthermore, Reiser (1940) found that during glucose absorption the inorganic phosphorus content of the intestinal mucosa was decreased, while that of the ester phosphorus was increased.

The Höbers (1936) found that the absorption of various amino acids was faster than their molecular mobility would indicate, when compared with other nitrogenous organic compounds, and concluded that some process other than simple diffusion was active in absorption of amino acids.

The present experiments were undertaken to determine to what extent, if any, these apparently active processes involved in the absorption of glucose and amino acid are affected by oxygen want.

METHODS. Pairs of dogs of as nearly the same weight as possible were used, one dog of a pair as a control and the other subjected to anoxia in a low pressure chamber. Partial pressures of oxygen used were 117, 94, 80, 63 and 53 mm. Hg, corresponding to altitudes of 8,000, 14,000, 18,000, 24,000 and 28,000 feet respectively.

Anesthesia was induced with ether and continued with sodium barbital, 220 mgm./kgm. A loop of intestine was prepared which consisted of the ileum and most of the jejunum. Loops in pairs of dogs were measured to be the same length. When the substance to be absorbed was glucose, the loop was washed with isotonic saline; for glycine absorption, it was washed with isotonic glucose. The intestine was gently stripped between the fingers to remove all washings.

Glucose absorption: Isotonic glucose solution (5.4 per cent) at 38°C.

[1] Aided by a grant of the Ella Sachs Plotz Foundation.

was placed in the loop, in quantity sufficient to fill but not distend it; it was then tied off and returned to the peritoneal cavity. The experimental animal was placed in a low pressure chamber for ninety minutes, the control being kept at atmospheric pressure for the same time. The solution remaining in the intestine was then removed and measured. A sample was diluted and analyzed for glucose by the method of Folin and Wu.

Glycine absorption: The procedure was the same as above, using isotonic glycine (2.3 per cent), which was allowed to remain in the loop for thirty minutes. Analysis for glycine was by Danielson's modification of Folin's method (Danielson, 1933).

At least eight, usually ten or more dogs were used at each barometric pressure for each substance. Nearly all the experimental animals were

TABLE 1

Effect of anoxic anoxia on the absorption of glucose and glycine

	OXYGEN TENSION																	
	155 mm. (Control)		117 mm.			94 mm.			80 mm.			63 mm.			53 mm.			
	Approximate altitude																	
	800 ft.		8,000 ft.			14,000 ft.			18,000 ft.			24,000 ft.			28,000 ft.			
	Number of animals	Per cent absorption	Number of animals	Per cent absorption	P*	Number of animals	Per cent absorption	P	Number of animals	Per cent absorption	P	Number of animals	Per cent absorption	P	Number of animals	Per cent absorption	P
Glucose	51	66.2	8	59.3	0.39	9	59.9	0.43	10	64.1	0.77	10	65.1	0.89	25	74.5	0.09
Glucose solution	51	58.2	8	51.4	0.48	9	43.7	0.14	10	53.9	0.62	10	51.9	0.51	25	68.1	0.09
Glycine	68	56.1	10	60.6	0.49	9	55.1	0.88	20	61.6	0.25	20	62.1	0.18	10	39.4	0.010
Glycine solution	68	47.6	10	52.3	0.51	9	45.4	0.78	20	54.8	0.19	20	53.8	0.25	10	30.3	0.019

* Probability of the difference from the control occurring by chance. Should be less than 0.05 to be significant (Fisher, 1932).

paired with controls, which resulted in large control series for both glucose and glycine.

RESULTS. The results obtained are summarized in table 1. It can be seen that both glucose and glycine are absorbed from isotonic solution faster than the water in which they are dissolved. The rate at which glucose is absorbed is depressed slightly at the relatively higher pressures used, practically unchanged at intermediate ones, and increased at the lowest (oxygen tension, 53 mm. Hg, a simulated altitude of 28,000 ft.). None of these changes is statistically significant, although the increase in absorption at 53 mm. Hg oxygen tension is nearly so.

The absorption of fluid roughly parallels the absorption of glucose, except at 94 mm. Hg oxygen tension, where it is depressed, though not significantly, more than glucose absorption.

The rate at which glycine is absorbed is essentially unchanged until an oxygen tension of 53 mm. Hg is reached, where it is significantly depressed. Again the fluid absorption roughly parallels the absorption of the solute, at a somewhat slower rate.

Discussion. Since the increase in absorption of glucose at 53 mm. Hg oxygen tension was quite marked but not statistically significant, an attempt was made to determine whether or not it was genuine.

Originally ten dogs had been subjected to this pressure; more experiments were done; but when a total of twenty-five dogs had been used without any important change in the results, the attempt was abandoned. After the first ten experiments, Fisher's formula showed seven chances in a hundred for the results occurring by chance. When the series of twenty-five were analyzed, the result was nine chances in a hundred. It is not practical to work with barbitalized dogs at any lower oxygen tension, as at 53 mm. Hg about one fourth of the dogs used die before the experiment can be completed. However, there is certainly no decrease in absorption at 53 mm. Hg partial pressure of oxygen.

Colowick, Welch and Cori (1940a) found that in kidney extracts oxidation of a dicarboxylic acid is necessary for phosphorylation of glucose. They further state (1940b) that phosphorylation precedes this oxidation, the latter process being necessary for the continuance of the reaction. They found this to be true also for brain extract.

If absorption of glucose by the intestinal mucosa is dependent on phosphorylation, it is rather surprising, in view of the above related facts, that anoxia does not retard its absorption even in the group of animals subjected to very severe degrees of anoxia. It appears likely, therefore, that phosphorylation in the intact mucosa may depend on a somewhat different mechanism than that investigated by Colowick et al. Gill and Lehman (1939), for instance, report that the formation of the Robison ester from glycogen is inhibited by oxidizing agents and increased by reducing agents.

The marked depression of the absorption of glycine at 53 mm. Hg partial pressure of oxygen suggests the possibility that an oxidative process may be directly involved in the absorption of glycine; certainly it appears that the process is a different one than that involved in the absorption of glucose.

An interesting point is the close parallelism between the absorption of these substances and the water in which they are dissolved. It appears that anything which alters the rate of absorption of the solute, at least when it is present in an isotonic solution, alters in a similar manner the absorption of the solvent. We have noticed this phenomenon in working with substances other than glucose and glycine.

In studying factors affecting absorption, the question always arises as to whether or not changes in the circulation are involved. There seems to be little doubt that cardiac output is increased in anoxic anoxia (Har-

rison et al., 1927; Strughold, 1930), but whether or not there is increased blood flow in the splanchnic area does not seem to have been determined. In the experiments herein reported, since anoxia produced different effects on the absorption of different substances under the same conditions, it is believed that circulatory changes were not an important factor.

SUMMARY

1. Anoxia up to and including 53 mm. Hg partial pressure of oxygen does not alter significantly the absorption of glucose from the small intestine of the dog.

2. Anoxia at 53 mm. Hg oxygen tension, but not higher partial pressures, significantly depresses the absorption of glycine.

3. The experiments suggest that possibly an oxidative process is directly involved in the absorption of glycine. The significance of the lack of effect of anoxia on glucose absorption is discussed.

REFERENCES

COLOWICK, S. P., M. S. WELCH AND C. F. CORI. J. Biol. Chem. **133:** 359, 1940a. J. Biol. Chem. **133:** 641, 1940b.

CORI, C. F. J. Biol. Chem. **66:** 691, 1925.

DANIELSON, I. S. J. Biol. Chem. **101:** 505, 1933.

FISHER, R. A. Statistical methods for research workers. Oliver and Boyd, London, 1932.

GILL, P. H. AND H. LEHMAN. Biochem. J. **33:** 1151, 1939.

HARRISON, T. R., C. P. WILSON, D. W. NEIGHBORS AND C. PILCHER. This Journal **83:** 275, 1927.

HÖBER, R. AND J. HÖBER. Proc. Soc. Exper. Biol. and Med. **34:** 486, 1936.

REISER, R. J. Biol. Chem. **135:** 303, 1940.

STRUGHOLD, H. This Journal **94:** 641, 1930.

VERZAR, F. AND E. J. MCDOUGALL. Absorption from the intestine. pp. 120 ff. Longmans, Green and Co., New York, 1936.

WILLBRANDT, W. AND L. LASZT. Biochem. Ztschr. **254:** 398, 1933.

THE COLLAPSE FACTOR IN THE MEASUREMENT OF VENOUS PRESSURE

The Flow of Fluid through Collapsible Tubes[1]

J. P. HOLT

From the Department of Physiology, University of Louisville School of Medicine, Louisville, Ky.

Accepted for publication July 16, 1941

If veins were rigid tubes, then a change in mean right auricular pressure would cause a corresponding change in the peripheral venous pressure, provided the velocity of blood flowing along the veins remained constant. However, veins are not rigid, but collapsible, and it has been shown by Lyon, Kennedy and Burwell (1938), and by Holt (1940), that peripheral venous pressure, referred to the level of the heart as zero, is increased when the vein is above heart level because the veins collapse. Carrier and Rehburg (1923) have also shown that the collapse of peripheral veins and capillaries may maintain capillary pressure at a high level when the capillary is above heart level.

Since veins are collapsible tubes, and the pressure in the right auricle is generally agreed to be subatmospheric, and there is a small positive tissue space pressure around the veins tending to collapse them, it was thought that changes in mean right auricular pressure might not affect peripheral venous pressure because the veins just before entering the chest might be partially collapsed.

Methods and results. When the veins entering the upper end of the thoracic cage were dissected out in the living dog, they were seen to be normally partially collapsed or to dilate and collapse synchronously with respiration. When the animal breathed air which was under a negative pressure the veins were seen to collapse more completely, and when air under a positive pressure was breathed the veins became dilated. The inferior vena cava was seen to collapse, after dissecting the liver away from it, when air under a negative pressure was breathed, but was not seen to collapse with normal respiration.

In ten barbitalized dogs with the chest closed, and placed in the supine position, mean peripheral venous pressure was measured in the femoral,

[1] A preliminary report of this work was given at the meeting of the American Physiological Society in Chicago, 1941.

cephalic, or jugular vein by a modification of the method of Moritz and Tabora (1910). At the same time mean right auricular pressure was measured by means of a saline manometer connected to a cannula that passed into the right auricle by way of the external jugular vein. The venous pressures were referred to the level of the cannula tip in the right auricle as zero. In some cases peripheral venous and auricular pressure fluctuated several millimeters with each respiration; in these cases the pressures were read at the peak of inspiration and at the peak of expiration (fig. 1). The trachea was cannulated and connected to a breathing cham-

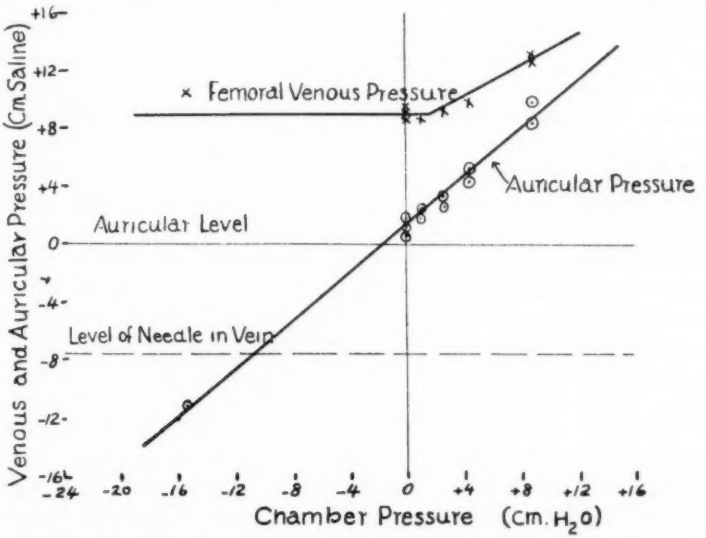


Fig. 1. The effect of changing the breathing chamber pressure on right auricular and femoral venous pressure. +, above atmospheric pressure. −, below atmospheric pressure.

ber in which the pressure was varied between twenty centimeters of water above atmospheric and twenty centimeters below. The chamber was built like a spirometer and had a volume between twelve and fifty liters in different experiments. The spirometer was ventilated with fresh air at a rate of fifteen liters per minute. The dog breathed from the chamber continuously, and when the pressure in the chamber was changed to any positive or negative value five minutes or longer were allowed before the venous and auricular pressure readings were taken. When the chamber pressure was increased the intra-thoracic pressure was increased and auricular pressure rose; when the chamber pressure was decreased the intra-thoracic and auricular pressures decreased.

The results of a typical experiment measuring auricular pressure and femoral venous pressure are shown in figure 1. When the breathing chamber pressure was increased the auricular pressure and peripheral venous pressure increased. When the chamber pressure was decreased the auricular pressure decreased but the peripheral venous pressure did not change.

Similar results were obtained on the jugular and cephalic veins with the exception that when the cephalic vein was used a rise in auricular pressure of a few centimeters of saline caused no increase in cephalic venous pressure, but a further rise in auricular pressure caused an increase in cephalic pressure. With the dog in the standing position results similar to those obtained on the femoral vein were obtained on the femoral and cephalic veins with the exception that a slight decrease in auricular pressure generally caused a decrease in peripheral venous pressure, but on further lowering auricular pressure the peripheral venous pressure remained constant. In all of the experiments described the vein was held below heart level. If the vein was held above heart level auricular pressure had to be increased several centimeters of saline before there was any rise in the peripheral venous pressure.

The maintenance of a high peripheral venous pressure when the auricular pressure was low might be explained by an increased rate of flow along the veins resulting from an increased cardiac output when the intra-thoracic pressure was low. In order to rule out this possibility a portion of the venous system of a dead dog was perfused with saline. The brachial, axillary, subclavian and innominate veins on one side, and the superior vena cava were dissected out, left in place, and all side branches entering these veins were tied. This left one large vein, with no open side branches, extending from the antecubital space to the right auricle. The brachial vein was cannulated in the antecubital space and the superior vena cava cannulated at the level of the right auricle. This system was perfused with saline, under a constant head of pressure, through the peripheral end of the brachial vein. The pressure in the peripheral brachial vein and in the superior vena cava was measured. With subatmospheric pressures in the superior vena cava the results were comparable to those obtained in the experiments on the femoral vein in the living dog. A similar experiment was performed on the inferior vena cava with like results. Thus it was shown that it was not necessary for the rate of flow along the veins to increase in order to maintain a constant peripheral venous pressure when the auricular pressure was greatly decreased.

In order to study how the collapse of veins might affect peripheral venous pressure a model (fig. 2) was set up using thin walled rubber tubes to represent veins or using the excised jugular vein of the dog. Water flowed from the Mariot bottle reservoir along heavy walled rubber tubing to a section of collapsible tubing and out through more heavy walled rubber tubing.

The pressures, P_1, above the collapsible segment, and P_2, below the collapsible segment were measured simultaneously with the rate of outflow. The collapsible segment was surrounded by a glass jacket in which the pressure was varied at will. The factors controlling the rate of flow of water through the collapsible tubes were studied by changing one of the above pressures, keeping the other two pressures constant, and measuring the rate of outflow into a graduate cylinder. In this system, when the outlet tube was lowered to a certain point, the pressure at P_2 became subatmospheric; thus P_2 corresponded to the auricular pressure, and P_1 to the peripheral venous pressure in the dog, while the jacket pressure corresponded to the tissue space pressure.

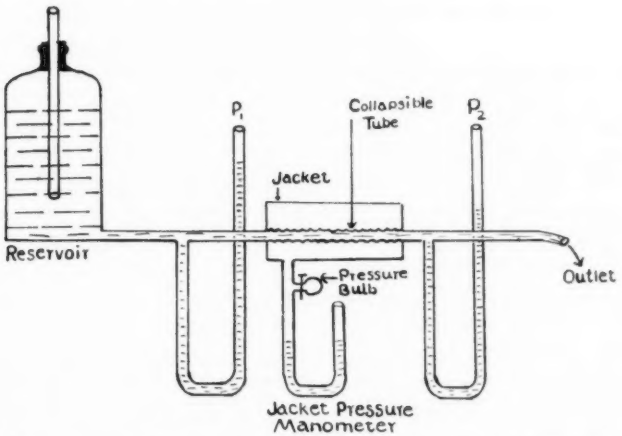


Fig. 2. Model used for studying the flow of water through collapsible tubes

The effect on P_1 of changing P_2 is shown in table 1 A. When P_2 was above jacket pressure (atmospheric in this case) and the tube was dilated, lowering P_2 caused a decrease in P_1, and this continued until P_2 became slightly subatmospheric at which point the tube collapsed. Further decrease of P_2 caused no change or a slight increase in P_1.

The effect of changing P_2 on the rate of outflow is shown in table 1 A. When P_2 was above atmospheric pressure and the collapsible tube was open, lowering P_2 caused an increase in the rate of outflow until P_2 became slightly subatmospheric at which point the collapsible tube started to collapse. As the tube started to collapse it began to pulsate and as P_2 was further lowered the rate of pulsation became more rapid. Finally when P_2 was lowered still further the pulsation apparently stopped and the tube remained partially collapsed. Lowering of P_2, once the tube had begun to

pulsate or to collapse, caused no change in the rate of outflow or a slight decrease.

The effect of changing P_1 on the rate of outflow with the tube open and again with the same tube partially collapsed, is shown in table 1 B. As P_1 increased the rate of flow increased in the open tube and in the partially collapsed tube. In both cases the increase in rate of flow was shown to be a linear function of the increase in P_1 when the data were plotted on graph paper.

TABLE 1

Flow of water through partially collapsed and open tubes

A						B						C		
						Open tube			Partially collapsed tube					
P_1	P_2	P_1-P_2	Flow	Tube	R	P_1	Flow	R	P_1	Flow	R	*J.P.*	Flow	R
cm. H_2O	*cm. H_2O*	*cm. H_2O*	*cc./ min.*			*cm. H_2O*	*cc./ min.*		*cm. H_2O*	*cc./ min.*		*cm. H_2O*	*cc./ min.*	
23.7	23.4	0.3	31.5	Open	0.0095	7.3	74	0.012	19.45*	63	0.76	22.3*	30	1.66
14.3	13.9	0.4	36.0	Open	0.011	8.0	182	0.0088	21.0	146	0.34	21.5	75	0.66
0.7	0.2	0.5	45.0	Open	0.011	8.85	276	0.0089	21.6	200	0.25	21.0	98	0.508
0.0	−2.4	2.4	45.5	Pulsating	0.052	9.50	372	0.0083	22.8	279	0.18	20.4	122	0.408
0.3	−12.1	12.4	45.5	Pulsating faster	0.27	10.80	480	0.009	24.0	360	0.14	19.9	175	0.284
1.0	−45.4	46.4	44.4	Collapsed, no pulse	1.04									
1.6	−105.4	107.0	44.0	Collapsed, no pulse	2.43									

A, effect on P_1 and on the rate of flow through the jugular vein of changing P_2. B, effect of changing P_1 on the rate of flow through a thin walled collapsible rubber tube 7 mm. in diameter (in the open tube P_2= 6.4 cm. of water, jacket pressure = atmospheric; in the partially collapsed tube P_2 = −28.5 cm. of water, jacket pressure = 19.9 cm. of water). C, effect of changing the jacket pressure on the rate of flow through the partially collapsed rubber tube (P_1 = 21.3 cm. of water, P_2 = −28.5 cm. of water). *J.P.*, jacket pressure. R resistance. −, minus.

* It took approximately 1 cm. water pressure to overcome the elasticity of the rubber tube and to collapse it, thus the jacket pressure is slightly higher than P_1 here.

The effect of changing the jacket pressure on the rate of flow when P_1 and P_2 were kept constant is shown in table 1 C. As the jacket pressure decreased the flow increased.

The fact that the rate of flow did not increase when P_2 was decreased, once the tube was partially collapsed (table 1 A), means that the resistance to flow through the partially collapsed tube increased as P_2 decreased. The resistance to flow may be calculated from the data in table 1 using the conventional formulation, $R = \frac{P_1 - P_2}{\text{Flow}}$. With the viscosity constant, as is the case in these experiments, the increase in R as the tube collapses must be caused by a decrease in the cross-sectional area of the collapsed segment, to an increase in turbulence, to an increase in the length of the

partially collapsed segment, or to a combination of these factors. No increase in the length of the partially collapsed segment was detected.

Table 1 A shows that in the partially collapsed tube the resistance increased as P_2 decreased, and table 1 B that the resistance decreased as the flow increased and as P_1 increased, and table 1 C that the resistance decreased as the jacket pressure decreased.

The length of the collapsible tube had little effect on the flow, since similar results were obtained on a collapsible tube 80 cm. long and on another 2 cm. long. When the longer tube collapsed the collapsed segment was always at the downstream 1 or 2 cm. of the tube, the rest of the tube remained open unless the flow through the system was very small. It appears that it was only necessary for the collapsible tube to be long enough and relaxed enough to collapse to give the effects of collapsible tubes described above.

The pressure: flow graphs that were plotted from the data in table 1 B were straight lines, whereas in experiments on smaller tubes, such as the jugular vein, the pressure:flow graphs were smooth curves convex toward the flow axis. This apparently is the result of the abrupt change in cross-section, which occurs at the point where the cannula tips are tied into the vein, causing the flow to be turbulent (Dodge and Thompson, 1937). However, qualitative results similar to those described in table 1 were obtained on these tubes.

Discussion. The fact that peripheral venous pressure remained constant when auricular pressure was decreased greatly and in some cases when auricular pressure was increased a small amount may have been caused in part by the change in auricular pressure being associated with a change in cardiac output and with a change in the rate of flow of blood along the veins. However, since the veins were seen to collapse, and since similar results were obtained on the excised jugular vein in a model, and on the dead dog's venous system acting as a model, it would appear that the collapse of the veins near the heart was an important factor in maintaining the peripheral venous pressure normal when auricular pressure changed.

Since auricular pressure may change independently of a change in peripheral venous pressure, it appears that the usual clinical measurement of venous pressure may give little indication of the pressure in the right auricle.

In the collapsible tubes studied here, the resistance to the flow of water decreased as the head of pressure, P_1, increased and as the jacket pressure decreased. Lowering the pressure on the downstream side of the collapsible tube increased the resistance to flow. Thus collapsible tubes differ from rigid tubes in that the resistance to flow remains constant in rigid tubes as the pressure-drop across the tube changes (so long as the flow is not

turbulent), while in collapsible tubes the resistance changes as the pressure-drop across the tube changes. Although the increased resistance to flow offered by the partially collapsed tube might be caused in part by an increase in turbulence at the partially collapsed segment, it seems certain that part of the increased resistance is caused by a decrease in cross-section of the segment, since the cross-section is observed to decrease in size when the tube collapses and appears to decrease still further the more the tube becomes collapsed.

It should be noted that in the graphs plotted from the data in table 1 B the pressure:flow line was steeper in the collapsed tube than in the open tube, that is, it took a greater increase of P_1 in the partially collapsed tube than in the open tube to cause a given increase in flow. The pressure: flow lines became steeper as the collapsible tube became more collapsed, i.e., at higher jacket pressures. This was the case in both the jugular vein and the thin walled rubber tubes. The reason for the greater slope of the pressure:flow line when the jacket pressure is higher is not clear.

Since the collapsible tubes studied show pulsation in the early part of the collapsing process, there is the possibility that some part of the venous pulse seen in the veins entering the chest may be caused by this type of pulsation. Also, since the veins entering the chest do collapse when there are moderate negative pressures in the right auricle, and since it has been shown that decreasing the pressure on the downstream side of a partially collapsed tube does not increase the rate of flow through the tube, it seems that this collapse of the veins entering the chest may be a mechanism which insures a normal flow of blood into the heart but prevents over-filling of the heart and intra-thoracic vessels when large negative pressures are present in the chest as in Müller's experiment or when a deep inspiration is taken.

SUMMARY

Right auricular and peripheral venous pressures were measured in dogs breathing from a chamber in which the pressure varied between 20 cm. of water above atmospheric and 20 cm. below. It was shown that when auricular pressure was decreased greatly and in some cases when auricular pressure was increased slightly the peripheral venous pressure remained constant. In most cases when auricular pressure increased the peripheral venous pressure was increased.

The flow of water through collapsible tubes such as the jugular vein of the dog was studied in a model. When fluid is flowing through a partially collapsed tube, increasing the pressure on the upstream side of the partially collapsed segment decreases the resistance to flow through the collapsible segment and increases the rate of flow, whereas lowering the pressure on the downstream side of the collapsible segment increases the resistance to flow through the collapsible segment and either does not change the rate of

flow or decreases it slightly. An increase in the jacket pressure around the collapsible tube increases the resistance to flow through the collapsible segment and decreases the rate of flow.

As a collapsible tube, having fluid flowing through it, starts to collapse it pulsates and as it becomes more collapsed the pulsation increases in rate on further collapse the pulsation apparently disappears.

The length of the collapsible tube is not important in controlling the length of the partially collapsed segment. It appears to be only necessary that the tube be long enough and relaxed enough to collapse in order to give the results described, and any length of collapsible tube greater than this length merely acts as a dilated or rigid tube.

REFERENCES

CARRIER, E. B. AND P. B. REHBERG. Skand. Arch. Physiol. **44:** 20, 1923.

DODGE, R. A. AND M. J. THOMPSON. Fluid mechanics. McGraw-Hill Book Co., 1937.

HOLT, J. P. This Journal **130:** 635, 1940.

LYONS, R. H., J. A. KENNEDY AND C. S. BURWELL. Am. Heart J. **16:** 675, 1938.

MORITZ, F. AND D. VON TABORA. Deutsch. Arch. f. Klin. Med. **98:** 475, 1910.

EFFECT OF GELATIN UPON MUSCULAR WORK IN MAN

PETER V. KARPOVICH AND K. PESTRECOV[1]

From the Department of Physiology, Springfield College

Accepted for publication June 19, 1941

Ray et al. (1939) claimed that gelatin feeding caused an increase of 37 to 240 per cent in the amount of work performed by male subjects. Since their experiment lacked controls, and the effect of training was underestimated, it seemed of interest to undertake further experimentation with gelatin feeding.

Five series of tests were performed. *Controlled diet:* 1, county jail inmates, bicycle riding; 2, campers, swimming. *Non-controlled diet:* 3, YMCA members, heavy-weight lifting; 4, YMCA members, wall-weight pulling; 5, college students, bicycle riding.

The reasons for using groups with non-controlled diet were: 1, it was desirable to repeat the experiment in a manner approximating the procedure of Ray and his co-workers, and 2, it was impossible to control the diet of some men beyond a request that they remain on their usual diet.

Experiments with county jail inmates. Twelve men, free from disease, were selected from a large number of volunteers. Eleven of the men were white and one was colored. The ages varied from 18 to 50 years.

The whole experiment may be subdivided into three periods:

1. Four weeks (one hour a day, five days a week) of building-up exercises for the legs and back muscles, because some of the men were not fit to work on the ergometers due to long enforced inactivity.

2. Five weeks of preparatory work on ergometers, with a gradual increase in rate of work starting from 0.06 H.P. The men worked five days a week (Saturdays and Sundays were excluded).

3. Period of actual experimentation, which varied from six to twelve weeks. The rate of work varied for different men from 0.159 to 0.261 H.P. (table 1). Work started after breakfast and continued as long as the subject could maintain a predetermined number of pedal revolutions per minute. When the riding continued for several hours, a subject might dismount and spend half a minute in going to the toilet (located in the experimentation room), but this did not happen for every subject every day.

Bicycle ergometers were used. Resistance was supplied by an automo-

[1] Now with the Scientific Bureau, Bausch & Lomb Optical Co., Rochester, N. Y.

bile brake lining around the fly-wheel. The upper end of the brake belt was attached to a spring scale with one ounce divisions, and weights were attached to its lower end. For an easier control of resistance which fluctuated occasionally, a simple device shaped like a trident was suspended by the middle prong from the brake cord. At least one pound in ounce weights was placed on its outer prongs; when the brake resistance fluctuated, some weights were either removed or added as the case might be. Hexagonal nuts each weighing one ounce were found to be convenient for this purpose.

TABLE 1

The essential data for the prison inmates working on the bicycle ergometers

The number of pedal revolutions per minute was 70 in all cases except that of no. 7, for whom it was 60 r.p.m. A rather high initial riding time and work are due to five weeks of preparatory training on the ergometers; during the fifth week the same rate of work was used as indicated on the table, but during this period the men rode from two to three times daily without reaching a point of exhaustion.

SUBJECT NO.	AGE	HEIGHT	WEIGHT	RATE OF WORK		WORK DONE IN FT.-LBS. PER DAY		RIDING TIME		PER CENT OF IMPROVEMENT
				Ft.-lbs. per min.	Horsepower	Initial	Maximal	Initial	Maximal	
			lbs.					*hrs. min.*	*hrs. min.*	
1	33	5′ 9″	170	6000	0.182	180,000	2,160,000	0 30	6 00	1100
2	41	5′ 6″	136	6000	0.182	240,000	576,000	0 40	1 36	140
3	44	5′ 7″	144	6000	0.182	72,000	1,896,000	0 12	5 16	2533
4	20	6′ 0″	178	7150	0.217	143,000	1,787,500	0 20	4 10	1150
5	28	5′ 2″	134	6000	0.182	60,000	1,290,000	0 26	3 35	727
6	24	5′ 11″	160	7000	0.212	140,000	1,680,000	0 20	4 00	1100
7	18	5′ 8″	121	5600	0.170	156,800	196,000	0 20	0 35	75
8	22	5′ 8″	174	6000	0.182	132,000	750,000	0 22	2 05	468
9	50	5′ 11″	184	5250	0.159	168,000	840,000	0 32	2 40	400
10	27	5′ 6″	140	7500	0.227	37,500	1,675,500	0 05	3 46	4420
11	23	5′ 11″	148	7150	0.217	228,800	2,659,800	0 32	6 12	1062
12	33	5′ 7″	204	8600	0.261	817,000	1,548,000	1 35	3 00	89

Nine ergometers were used simultaneously. Each was equipped with a speedometer and distance meter. A metronome was also used to double-check the rate of pedaling.

Diet. All inmates were on the same basic diet, although the amount of bread consumed varied somewhat, since they could have all the bread they wanted. Subject 2 received a small additional quantity of milk daily to provide more liquid in his diet because of dental work in progress.

Work on jail inmates started with body-building exercises which continued for four weeks and comprised the first period of the experiment. After this, the preparatory work on ergometers began, all subjects starting

without gelatin. Three and a half weeks later 6 of the men were given 32 grams of gelatin in grapefruit juice, and 6 others were given an equal amount of a cereal (farina) in the same way. The latter was called "concentrated gelatin" to explain the insolubility of farina in the grapefruit juice, and to assure these men that they were merely getting another kind of gelatin.

The result of this change in diet was immediate. Every subject showed an improvement within an hour after ingestion of either gelatin or cereal, indicating that the effect was purely psychological.

The performance continued to increase so rapidly that it became necessary to increase the loads to the values shown in table 1 in order to keep the riding time within reasonable limits. We also discontinued gelatin feeding and put every man on farina. This part of the experiment *perforce* had to be regarded as a part of the preparatory work, constituting the second period.

One week was allowed the men to become familiar with the new heavier loads; after this we started the third, final period of the experiment. All men worked on farina diet from four to seven weeks, then they were given gelatin. This diet was continued for some time and then gelatin was again withdrawn, and in its place farina, fruit juice or neither was given. In no case did the men know when they were getting gelatin or a substitute.

Results. From table 1 it may be seen that increase in performance varied from 75 to 4420 per cent. The latter figure may appear to be large, but as a matter of fact it should have been larger. During the preliminary training on the ergometer most of the men could not last more than ten minutes with lighter loads. In order to check the difficulties of the ergometer work we tried it ourselves. It took us two weeks to pass the five minute mark at 0.182 H.P.

Figure 1 presents four typical curves of performance. Subject 11 seemed to improve markedly with the addition of gelatin to his diet. Yet it would be unwarranted to conclude that this was a gelatin effect, because subject 4, who had no gelatin, showed an identical gain at the same time; and subjects 5 and 7 were not affected although gelatin was given to them. A sudden drop in the performance of the subject 4 was a result of a knee injury. The performance of the subject 5 continued to improve after the withdrawal of gelatin and the substitution of cereal. But when cereal, known to him as "concentrated gelatin" was withdrawn, performance immediately dropped, and the subject complained that he was getting too tired without the "gelatin."

It is impossible to note any special effect of gelatin upon the work output. Addition of gelatin to the diet did not improve performance, nor did the withdrawal of gelatin make it worse. All increase in performance was undoubtedly a result of training.

Validity of the test. While work of long duration may be cut short by psychological factors, as suggested by Simonson and Sirkina (1933), special efforts were made to elicit maximal performances. The prisoners preferred exercise at an hourly rate of pay to idleness in their cells. A competitive spirit was aroused by having them work side-by-side. At the end there was subjective and objective evidence of exhaustion. One educated and intelligent subject remarked that he kept on until he became dizzy. At the end of a five-hour ride he was unable to talk and his reactions were

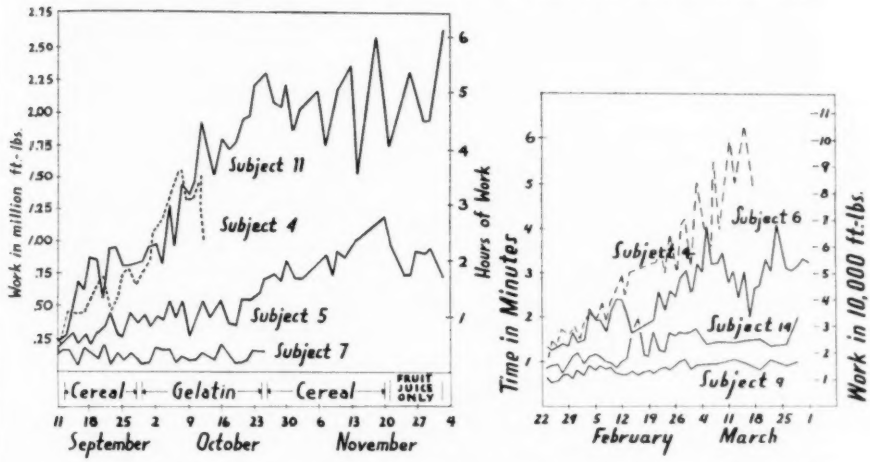


Fig. 1. Typical curves of prolonged work on bicycle ergometers. Subjects—jail inmates. Subjects 4 and 11—0.217 H.P.; subject 5—0.182 H.P. and subject 7—0.170 H.P. Subjects 5, 7 and 11 received gelatin as indicated on the graph. Subject 4, whose curve follows that of no. 11, received no gelatin. The drop in his curve was due to a knee injury.

Fig. 2. Typical curves of short intensive work on bicycle ergometers. Rate of work—0.506 H.P. Pedal revolutions—117 per minute. Subject 4, whose performance is the best, received no gelatin. A drop in performance in curves 6, 14 and 9, which occurred between March 4 and 18, was the effect of college term examinations.

slow. It was noted that after a rest in a reclining position some subjects had to lift their legs by hand as they arose from the bed. These observations indicate to us that physiological rather than psychological factors were responsible for the steady increases in performance.

Metabolism studies. The metabolism of five men was studied after ten weeks of training. Two of the men were on gelatin at that time and three were not. Metabolism was tested: 1, lying; 2, sitting on bicycle; 3, pedaling bicycle without load (free wheeling); 4, pedaling bicycle with full load; and 5, recovery after exercise. During the work with full load, expired air was collected for 4 to 5 minutes each half-hour. The Douglas-Haldane

method was used. No relation between metabolism data (table 2) and gelatin feeding was noticed.

Source of energy. The appetite of all men increased with the increase in riding time. Since bread was supplied in liberal amounts at each meal, the subjects ate it in large quantities. When, for instance, subject 11 passed five hours of riding, he would take 12 to 14 slices of bread with his breakfast, 14 to 19 slices of bread with lunch and a pound loaf with his supper; he was eating from one to two pounds more of bread than before. Assuming that a pound of bread supplies 1180 calories, an extra supply of 1180 to 2360 calories was provided. The amount of net energy spent on the bicycle by subject 11 in five hours was 1716 calories; thus it is obvious that the principal source of *extra* energy for the prolonged riding was the

TABLE 2

Summary of data obtained in work metabolism tests

Subjects were jail inmates working on bicycle ergometers. In calculating the net amount of energy used and efficiency, free wheeling was taken for the base.

SUBJECT NUMBER AND DIET	O_2 CONSUMPTION PER MIN. DURING WORK	OXYGEN DEBT	RESP. QUOTIENT	WORK PER MINUTE	NET ENERGY USED PER 1000 FT.-LBS. OF WORK	NET ENERGY USED PER MINUTE	EFFICIENCY PER CENT OVER FREE-WHEELING
	liters	*liters*		*ft.-lbs.*	*Calories*	*Calories*	
1. Gelatin	1.9	2.32	0.96–0.98	6000	1.13	6.78	29
3. Gelatin	1.7	0.224	0.91–0.97	6000	1.05	6.30	31
4. No gelatin	2.3	2.70	0.91–0.98	7150	1.08	7.72	30
5. No gelatin	1.7	0.200	0.92–0.99	6000	1.07	6.42	30
11. No gelatin	1.4	2.00	0.97	7150	0.80	5.72	41

extra amount of bread consumed. This was also substantiated by his respiratory quotient of 0.97 during work.

Efficiency of work. For calculation of energy used for overcoming the brake resistance we accepted the metabolism at free-wheeling as the point of reference. With one exception, the efficiency of the men was about the same for all, varying from 29 to 31 per cent. The exception was subject 11 whose efficiency was 41.0 per cent. These figures lie within the range found by Benedict and Cathcart (1913). On our subjects it was impossible to notice any effect of gelatin feeding upon the efficiency. The high efficiency of subject 11 should be attributed to "individual difference."

Immediate effect of free wheeling upon work with full load. During the first two or three metabolic tests, we encountered a peculiar psychological effect which occasionally compelled us to discontinue the testing for that day. After 15 to 20 minutes of free wheeling, the subjects found it extremely hard to work with their ordinary load. Some of the men gave

up in 10 minutes, insisting that the weight had been doubled. They exhibited all the symptoms of extreme fatigue. After checking the weights and proving that they were the same as always, and after allowing a 10 to 15 minute rest, the men were able to resume their work; but on such a day they could never equal their expected riding time. In further tests we found that by a preliminary explanation and by encouragement it was possible to eliminate this factor and to make the men work as usual. Even then there was a certain critical point which seemed difficult to pass; however, after it had been passed, work seemed to be easy. This period varied from 10 to 30 minutes after beginning of the riding, being rather constant for each man. For instance, subject 1 had his critical period 25 to 30 minutes after the start.

College students. The subjects for this series of experiments were 16 Springfield College students, ranging in age from 18 to 26 years, and in weight from 139 to 184 pounds. The exercise consisted of riding on bicycle ergometers at a rate of 16,700 ft-lbs. per minute, or 0.506 H.P., and 117 pedal revolutions per minute. They worked five days per week, riding twice a day with a five-minute rest between the rides. After six weeks of experimenting it was observed that improvement in performance in most men was small. Upon the suggestion of D. B. Dill it was decided to introduce a change in testing procedure.

Four men, nos. 4, 6, 12 and 13, continued as before, but the other twelve men worked at the original rate of 0.506 H.P. only twice a week, and on the other three days they rode only once a day at a rate of 0.329–0.354 H.P.

Rate of work. Although the rate of 0.506 H.P. of work is quite large, some of the men, nevertheless, could keep up this rate for several minutes; the maximum was 6 minutes 18 seconds.

From the article by Ray et al. (1939) it is impossible to see the real work output which was used in their study. Evidently their ergometer had a poor efficiency, since an output of 60 watts (0.08 H.P.) was able to fatigue the men in a short time.

The rate of work of women subjects in the experiments of Hellebrandt, Rork and Brogden (1940) approximates that found in our investigation. With allowance for sex, an output of 220 to 290 watts (0.29 to 0.39 H.P.) is a large output for a woman.

Diet. The basic diet of the men could not be controlled outside of a request that they eat about the same during the course of the experiment. For control purposes, they were divided into several groups (see table 3).

Results. Examination of table 3 will show that the per cent of improvement in work varied from 49 to 334. The man who improved most received no gelatin at all. Unfortunately he had to quit the experiment at the end of the eighth week due to an urgent call from home.

We should like to call attention at this time to a possible error that may

TABLE 3

Time and per cent of improvement over the daily average work during the first week in college students

Subjects working on bicycle ergometers at 0.506 H.P. and 117 pedal revolutions per minute. The duration of the experiment was 10 weeks.

GROUP	SUBJECT NO.	RIDING PERFORMANCE		IMPROVEMENT
		Ave. 1st week	Maximum attained	
		seconds	*seconds*	*per cent*
1				
Gelatin throughout experiment	13	56	240	330
	14	52	122	135
	15	37	66	78
	16	38	77	103
Average		45.75	126.2	161.5
2				
Farina 4 weeks; gelatin 6 weeks	1	53	95	79
	2	42	73	74
	3	46	70	49
Average		47	79.33	67.33
3				
Blank 6 weeks; gelatin 4 weeks	5	26	63	142
	12	56	185	230
Average		41	124	186
4				
Farina throughout experiment (no gelatin)	6	80	246	208
	7	41	72	76
	10	37	88	138
	11	42	83	98
Average		50	122.25	130
5*				
Farina 4 weeks; blank 4 weeks (no gelatin)	4	87	378	334
6				
No gelatin; no farina	8	61	128	110
	9	37	63	70
Average		49	95.5	90

* Had to discontinue at end of eighth week for reasons not involving health.

occur in the interpretation of results obtained in experiments similar to ours if composite curves representing the average performance of the different groups are used. If by chance we used only twelve men, and groups 2 and 4 (see table 3) were absent, then this table could have been used as proof of a beneficial effect of gelatin, since the groups 1 and 3 give a higher per cent of improvement than the non-gelatin groups 4 and 6. Only by a comparison of the differences within each group does it become evident that the variation within a group is greater than any variation between groups.

The only conclusion that can be made is that gelatin had no effect upon the work capacity of the men in this experiment. This conclusion is in perfect agreement with the observation by Hellebrandt et al. (1940). Maison (1940) also found no effect of gelatin feeding in four subjects who exercised on a finger ergograph. On theoretical grounds his results may be disputed because of the smallness of the muscle group used. Even if glycine were beneficial, it could have been supplied to the finger muscles at the expense of the other groups. This is why we preferred exercise involving large groups of muscles. On the bicycles not only are the legs involved, but also the trunk muscles, and even the arm muscles are constantly being used.

Daily work done by the College Students is presented in the figure 2. The rate of work was 0.506 H.P. for all men, yet the rapidity and the degree of the improvement varied considerably. This was due to a great extent to a difference in muscular strength. Subjects 4 and 6 were among the strongest men in the group.

In the three lower curves (fig. 2) a depression may be observed between March 4 and 18. This coincided with the period of term examinations held between March 11 and 18. Of sixteen men only three were not affected by examinations.

After completion of a ten-week period subject 6, who belonged to a group not receiving gelatin, continued to work on the ergometer for two more months. At the end of this extra period, his riding time had reached 7 minutes, 30 seconds, which means a work output of 125,310 ft-lbs., an improvement of 463 per cent. His curve showed a continuous rise, but the experiment had to be discontinued because of college final examinations. However, this clearly indicates that the peak in training on the bicycle may not be reached even after 19 weeks of training.

It is interesting to compare these figures with those obtained by Henderson and Haggard (1925) on Olympic oarsmen. They estimated the rate of work done in breaking the world's record for a mile and a quarter in 5 minutes 51 seconds, as being equal to 0.57 H.P. By coincidence, subject 6 was a candidate for the Olympic contests in canoeing.

Performance during the second ride with 0.506 H.P. rate. No effect of gelatin could be observed on recovery from the first ride.

Lighter loads. Three days a week of riding with lighter loads (0.329–0.354 H.P.) did not affect the curve of performance obtained in riding two days a week at a rate of 0.506 H.P.

Experiments with summer camp boys. Thirty boys, ranging from 15 to 17 years in age, were selected in two summer camps. In each camp they were divided into two groups, one receiving 32 to 48 grams of gelatin daily, and the other receiving a non-gelatinous substitute. The rest of their diet was essentially the same for the boys in each camp. They swam daily (60 to 100 yds.) trying to better their time.

The experiments continued for 6 to 8 weeks. No difference was observed between the gelatin and non-gelatin group.

Experiments with heavy-weight lifters. Twelve heavy-weight lifters who were members of two YMCA organizations volunteered to take part in this experiment. They exercised two or three times a week for 10 weeks trying each time to beat their previous records in the nine standard types of weight lifting. The basic diet could not be controlled beyond a request that they use "approximately the same diet" throughout the experiments. The men were divided into two groups: gelatin (32 to 64 grams daily) and non-gelatinous substitute. No difference could be observed between the performance of these groups.

Experiments with wall-weight pullers. Exercises for the arms and shoulder retractors were used, with the height of elevation of the weights kept constant and the rate controlled by metronome. Six men (gelatin 32–64 grams daily, and no gelatin) exercised until they could not maintain the rate. This experiment proved unreliable due to erratic performances. However, no difference was noted in the performance of the two groups.

SUMMARY AND CONCLUSIONS

The effect of gelatin feeding upon muscular performance was tested on five groups of subjects, numbering 76 in all. Exercises used were: work on bicycle ergometers, swimming, weight-lifting and wall-weight pulling. Diet was either fully or partially controlled. Sham gelatin feeding by use of a non-gelatinous substitute was practiced with each group.

1. Twelve county jail inmates, 18 to 50 years of age, exercised on ergometers for from 17 to 22 weeks, 5 times a week. The rate of work varied from 0.159 to 0.261 H.P. Performance improvement ranged from 75 to 4420 per cent; the maximum riding time of one subject reached 6 hours 12 minutes, and the work done on that day was 2,659,800 ft-lbs. Diet was fully controlled. No effect of gelatin feeding could be observed.

2. Sixteen college students between 18 and 26 years of age exercised on bicycle ergometers five times a week for 10 weeks. Their rate of work was

0.506 H.P. Improvement in ten weeks was between 49 and 334 per cent, and in the case of one man who exercised for 9 extra weeks at 0.506 H.P. it reached 463 per cent; the maximum working time was 7 minutes 30 seconds. Diet was not controlled. No effect of gelatin feeding was observed.

3. Thirty campers, ranging from 15 to 17 years of age, swam 60 to 100 yards each day from 6 to 8 weeks. Diet was controlled. No effect of gelatin feeding could be observed.

4. Twelve weight lifters tried to break their previous records in nine standard positions. Diet was not controlled. No effect of gelatin could be observed in 10 weeks.

5. Six men exercised by pulling wall weights 3 days a week, attempting each time to increase the number of pulls. Diet was not controlled. No effect of gelatin could be observed.

6. At the same rate of work, the stronger men improved more than the weaker ones.

7. Metabolism of 5 jail inmates was studied. Efficiency of work varied from 29 per cent to 41 per cent. The maximum amount of net energy spent on daily exercise reached 2441 Calories. The *extra* energy for this work was supplied by the extra amount of bread consumed. No effect of gelatin could be observed in the metabolic tests.

8. College term examinations caused a drop in performance of most of the subjects.

9. A psychological effect of gelatin and sham feeding was observed in jail inmates. The first day they received either gelatin or a non-gelatinous substitute, their performance noticeably increased before either of the substances could have been digested and assimilated.

We wish to thank Mr. Harold LeMaistre, of Sydney University, Australia, for help with metabolic tests of jail inmates.

REFERENCES

BENEDICT, F. AND E. CATHCART. Carnegie Inst. Washington, Publication no. 187, 1913.

HELLEBRANDT, F. A., R. RORK AND E. BROGDON. Proc. Soc. Exper. Biol. and Med. **43:** 629, 1940.

HENDERSON, Y. AND H. HAGGARD. This Journal **72:** 264, 1925.

MAISON, G. L. J. A. M. A. **115:** 1439, 1940.

RAY, G. B., J. R. JOHNSON AND M. M. TAYLOR. Proc. Soc. Exper. Biol. and Med. **40:** 157, 1939.

SIMONSON, E. AND G. SIRKINA. Arb. Physiol. **7:** 457, 1933.

ACUTE EFFECTS OF SPINAL CORD SECTION UPON THE PLASMA VOLUME AND BLOOD PRESSURE OF CATS UNDER ETHER ANESTHESIA

ASHER E. TREAT

From the Department of Physiology, College of Physicians and Surgeons, Columbia University

Accepted for publication June 22, 1941

It is well known that surgical or other interruption of spinal pathways is commonly followed by a fall in arterial pressure. The possibility of a change in blood volume occurring under these circumstances has received relatively little attention. Eppinger and Schürmeyer (2) reported a single experiment in which high spinal transection apparently reduced the blood volume of a dog from 1500 cc. to 907 cc. They considered this result as comparable to their findings in animals suffering from various forms of circulatory collapse. Nowak (10), using the congo red dye method, estimated the blood volume of cats before and during spinal anesthesia, and found an average increase of 3 cc. per kgm. body weight. His animals had undergone previous laminectomies and were anesthetized with barbiturates during the spinal block. Goldfarb, Provisor and Koster (3) concluded from data obtained by the brilliant vital red method on surgical patients that no significant blood volume change occurs during spinal anesthesia, although their values for percentage change in individual cases range from −11.9 to +15.7. The three papers just cited deal only incidentally with blood volume or plasma volume changes, and in none of them are the experimental conditions and methods described sufficiently to permit an evaluation of the results.

A correct interpretation of plasma alterations in animals undergoing acute interruption of cord pathways requires that account be taken not only of local modifications within the cord itself, but also of a variety of changes incidental to the experimental procedure. These include premedication, anesthesia, incidental operative trauma, respiratory disturbances, modified sympathetic activity, and a number of other factors. In chronic animals, the effects of muscular disuse and of change in dietary or other habits may be important. Moreover, animals surviving transection or total destruction of the spinal cord even for short periods may regain not only their pre-operative blood pressure levels, but also a limited reflex control of the circulation (6, 13). Accordingly, the writer decided

to use acute preparations, and, as will be seen, to eliminate certain of the disturbing factors. It was hoped that in this way any plasma volume changes which might occur could be referred with some confidence to the effects of cord transection as such. It must be emphasized, however, that the factor of anesthesia has not been eliminated in these experiments, and that the results are therefore applicable only to cats under the conditions described. Obviously the attempt to obtain control values and to transect

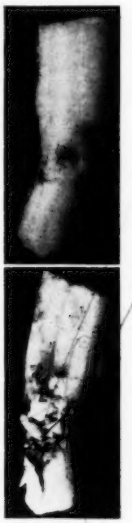


Fig. 1

Fig. 2

Fig. 1. Portion of spinal cord of cat 17, showing lesion. Above: dorsal view. Below: ventral view.

Fig. 2. Instrument for destroying portion of cervical cord. Inset on left: cross section through shaft of needle, showing plunger and long arm of wire loop. For further description, see text, p. 4.

the cords of unanesthetized animals could only lead to sympathetic activity far more disturbing than that occasioned by ether.

METHODS. The traumatic effects of laminectomy with attendant blood loss and tissue exposure were avoided by the use of a blind puncture instrument (fig. 2). The main part of this device was a four inch, thirteen gauge hypodermic needle. A J-shaped loop of no. 4 music wire was arranged within the shaft of the needle so that it could be anchored by its long arm outside the needle's head, while the short arm engaged a plunger the lowering of which caused the wire loop to emerge from the orifice of the needle and to spread to a diameter of 6 to 8 mm. With the animal under

ether anesthesia, this loop was spread within the substance of the spinal cord, and then rotated by a turning of the needle so as to reduce the cord to a pulp at the level of the puncture. Following the withdrawal of the loop by traction upon its long arm, a heated rod was plunged into the pulped cord through the same needle, in order to coagulate any fibers remaining intact, and, by cauterization, to reduce the danger of infection or of extensive internal bleeding. When the operation is performed at or above the level of the eighth cervical segment, only a slight nick in the skin is necessary for the introduction of the needle. The animal is placed upon its side, with the neck slightly flexed. . The neural spine of T_1 serves as a guide for the needle point as far as the ligamentum flavum. When this is reached, a quick thrust will generally send the needle point directly through the center of the cord. Thereupon the loop is spread, rotated, and withdrawn; then cauterization is applied as described above. The entire operation consumes less than two minutes. When a chronic preparation is desired, the method offers the advantage that the period of anesthesia may be kept exceedingly short. Adequate antisepsis may be attained merely by boiling the puncture instrument. Of six chronic spinal cats so prepared, none showed any sign of infection either in the cord or in the superficial tissues. Ten days to several months after the transection, the animals were killed with ether.

Disadvantages of this method include the following: *a*, the destruction of the cord is not invariably complete, and must be checked at autopsy; *b*, the lesion is never sharp and clean-cut, but contused and likely to be somewhat asymmetrical with reference to the long axis of the cord; *c*, the cord may be attacked only where it is not protected by overlapping bony arches—practically, in cats and dogs, the cervical region.

At frequent intervals during each of the experiments, the animal was examined for functional evidence of nervous transmission across the lesion. At no time did such evidence appear. After recovery from ether each cat showed complete volitional paralysis and anesthesia in the trunk and hind quarters. Breathing was entirely diaphragmatic. The nictitating membranes were widely extended. At the conclusion of each experiment, a portion of the cord including the lesion was removed, freed from its outer membranes, and examined grossly. When there was any reason to suspect that complete functional destruction had not been attained, the experiment was discarded. Figure 1 is a photograph of a cord (no. 17) showing a typical lesion. The specimen had been kept in 10 per cent formalin for several weeks.

Plasma volume determinations were made by the use of the dye T-1824, optical densities being estimated spectrophotometrically (4). A control sample of 1 cc. was taken from the right saphenous vein soon after anesthesia had been induced. Following the dye injection, and the cannulation

of the right femoral artery, eight 1 cc. samples were generally drawn from the left saphenous vein. Four of these samples were taken before the spinal transection, and four after, at intervals of about fifteen minutes. The first post-operative sample was usually obtained within five to ten minutes after the transection, the time chosen being that at which the blood pressure appeared to have reached its minimum value. Deviations from a control curve established by the first four dye samples gave the plasma volume fluctuations here interpreted as the result of the spinal section. These deviations were checked by comparison with serum protein and hematocrit determinations. Since the first post-operative sample commonly shows the maximum deviation from the control curve, the experimental change (see table 1) was reckoned from this value. The deviation of the last sample was taken as an index to the extent of return of the plasma volume to its control level.

Proteins were estimated refractometrically in the sera which had previously been used for the dye determinations, the calculations being based upon the factors given for dog serum by Neuhausen and Rioch (9). These data were used only for purposes of comparison with the dye curves, and therefore relative values only were required. Nevertheless, it was thought desirable to check the refractometric estimates against those made by some other method. Through the kindness of Dr. A. Graff, a comparison was made of the values obtained by the use of the refractometer with the results of microkjeldahl analyses, assuming a non-protein nitrogen content of 30 mgm. per 100 cc. The average serum protein concentration in five refractometer determinations was 6.85 per cent. Microkjeldahl analyses of the same sera gave an average protein concentration of 6.81 per cent. The greatest difference between the values given by the two methods for any one sample was 0.06 per cent. It can be shown that a change of 100 per cent in non-protein nitrogen affects the serum protein estimate by only 0.3 per cent. The refractometer estimates, therefore, are probably good approximations of the absolute values. Two hematocrit samples were taken for further comparison with the dye curve. One was included with the last pre-operative, and one with the first post-operative dye sample. The total amount of blood lost in sampling was about 10 cc.

The cats were starved for 18 to 20 hours. They were then weighed, placed under a bell jar and anesthetized with ether. Following the induction stages, the anesthesia was continued by the cone drop method. After preliminary sampling and injection of the dye, the right femoral artery was cannulated and connected to a Hürthle manometer. In one experiment (no. 9), through the kindness of Dr. H. Wiggers, blood pressure and pulse variations were recorded by means of an optical manometer. The attempt was made to regulate the depth of anesthesia and the heat exchange of the animal in such a way that blood pressure, respiration and

rectal temperature remained fairly constant during the hour or more of intermittent sampling prior to the transection of the cord. Difficulties with clotting experienced in preliminary experiments were met by using a

TABLE 1

Effects of cord destruction at C_3 in acute experiments under ether anesthesia

CAT	SEX	WEIGHT	HEMATOCRIT, PER CENT CELLS			MEAN ART. PRESSURE, MM. Hg			SERUM PROTEINS, MGM. PER CENT			PLASMA VOLUME, CC.			REMARKS
			Control	Postop.	Diff.	Control	Postop.	Diff.	Control	Postop.	Diff.	Control	Postop.	Diff.	
		kgm.													
1	♂	2.93	39.4	37.2	−2.2				6.3	6.0	−0.3	138	147	+9	Animal intended for survival
2	♂	3.96	27.0	24.9	−2.1	145	73	−72	6.2	5.9	−0.3				Lipemic serum
3	♂	2.92	31.7	30.5	−1.2	140	80	−60	6.3	6.0	−0.3	110	116	+6	
4	♀	3.13	39.6	37.1	−2.5	130	85	−45	6.4	6.4	0	119	128	+9	
5	♂	3.13	38.2	32.8	−5.4	115	75	−40	6.8	6.4	−0.4	148	154	+6	
6	♂	3.19	41.3	37.8	−3.5	160	120	−40	6.0	5.6	−0.4	118	122	+4	
7	♂	3.43	29	29	0	145	85	−60	6.0	6.0	0	151	151	0	
8	♂	3.52	43.2	38.7	−4.5	155	84	−71	5.9	5.3	−0.6	111	124	+13	
9	♂					175	95	−80							With optical manom.
Average					−2.67			−58.5			−0.29			+6.7	
Average as per cent of control					−7.79			−42.2			−6.7			+5.5	
10	♀	2.75	45.8	43	−2.8	105	60	−45	5.8	5.6	−0.2	115	123	+8	Atropinized
11	♀	2.59	35.5	29.2	−6.3	125	70	−55	6.5	6.1	−0.4	105	105	0	Atropinized
12	♀	2.36	28.7	26.4	−2.3	125	85	−40	6.8	6.8	0	141	141	0	Atropinized (Lactating and feverish)
13	♂	2.69	36.7	34.2	−2.5	120	75	−45	7.1	6.9	−0.2	138	142	+4	Atropinized
14	♀	2.36	48.1	44.8	−3.3	140	100	−40	6.1	5.9	−0.2	106	111	+5	Atropinized
15	♀	2.46	40.9	38.8	−2.1	130	130	0	7.0	7.0	0	93	93	0	Vagotomized
16	♂	3.70	42.6	38.9	−3.7	140	72	−68	6.8	6.1	−0.7	161	170	+9	Vagotomized
17	♂	3.30	44.4	40.3	−4.1	170	72	−98	6.6	6.0	−0.6	101	114	+13	Vagotomized
18	♀	3.21	35.2	33.5	−1.7	155	72	−83	6.1	5.9	−0.2	129	132	+3	Vagotomized (Pregnant)
19	♀					140	105	−35							
Average					−3.20			−50.9			−0.28			+4.7	
Average as per cent of control					−7.99			−31.2			−3.5			+3.4	

cannula of large bore, carefully coated with paraffin. If care was taken to avoid chilling the arteries with cold instruments, good sized cannulae could be introduced even into the arteries of small cats, and once the skin wound was closed with a clamp, a continuous record could be secured for long periods without clots.

Upon the destruction of the cord segment, the use of the anesthetic was discontinued except for brief control periods occasionally introduced after sampling had been completed, in order to demonstrate that ether withdrawal had not caused the observed change in blood pressure. A few experiments upon chronic spinal cats were also undertaken, in order to determine the effect of the administration and withdrawal of ether upon the plasma volume of such animals. The results are summarized in table 2. In some of the acute experiments, bilateral cervical vagotomy was performed shortly before the first dye sample was taken. In others, atropine was given intraperitoneally a half-hour before the induction of anesthesia. The dosage was such as to produce mild mydriasis, generally 0.04 mgm. per kgm. body weight.

TABLE 2

Effects of ether anesthesia upon chronic spinal cats

CAT	SEX	WEIGHT	DAYS SPINAL	DURATION OF ANESTHESIA	HEMATOCRIT, PER CENT CELLS			SERUM PROTEINS, MGM. PER CENT			PLASMA VOLUME, CC.			REMARKS
					Control	Ether	Diff.	Control	Ether	Diff.	Control	Ether	Diff.	
		kgm.		*min.*										
20	♂	2.27	4	89	33.7	34.6	+0.9	5.1	5.4	+0.3	133	120	−13	Excessive salivation
21	♂	3.14	11	64	30.4	27.7	−2.7	6.8	6.7	−0.1	167	167	0	
22	♂	2.39	6	72	30.4	27	−3	6.0	6.1	+0.1			0?	Plasma volume curve indecisive
23	♂	2.62	63	44	34.0	26.7	−7.3	6.9	6.8	−0.1	138	138	0	
1	♂	2.51	42	50	32.0	32.5	+0.5	6.7	6.1	−0.6	152	169	+17	
20	♂	2.10	28	54	30.0	24.5	−5.5	5.6	5.7	+0.1	135	135	0	Atropinized
23	♂	2.50	55	48	33.0	33.8	+0.8	6.4	6.4	0	114	114	0	Atropinized
1	♂	2.50	56	40	24.9	29.1	+4.2	6.7	6.4	−0.3	149	158	+9	Atropinized

RESULTS. The principal results are summarized in tables 1 and 2. The general circulatory changes which are usually associated with high spinal transections were observed with regularity. During the destruction of the cord segment, the mean femoral pressure underwent a transient rise which was sometimes very great and sometimes barely perceptible. In all experiments except one (no. 15), this initial rise was succeeded by a fall, the amount of which, in cats with intact vagi, averaged 42 per cent of the control value, and in vagotomized cats, 37 per cent of the control value. In most instances the minimum pressure was reached within five minutes after the operation, and was followed by partial recovery. In the animals with cut vagi, the blood pressure regained as much as two-thirds of the control value within one hour following the transection. When partial recovery occurred in the non-vagotomized cats, the extent was much less

in a corresponding interval. Previous section of the vagi also appeared to diminish the time required for recovery from ether.

During the primary rise in pressure which frequently attended the destruction of the cord segment, the heart rate was quickened. The increased rate was maintained throughout the falling phase of the pressure curve, during which time, also, the pulse pressure was usually much reduced. In many experiments transient cardiac arrhythmia appeared during the time of initial high pressure, but this vanished before the minimum pressure level was attained. Following the period of tachycardia, the heart rate gradually fell, and during most of the recovery period it averaged 15 to 20 per cent lower than the control value. With the exception of cat 15, this secondary slowing of the pulse occurred in the vagotomized animals as well as in the others. Cat 15 maintained an increased heart rate throughout the period of observation, and entirely escaped the fall in pressure, the only significant change being a moderate and fleeting rise as the cord was attacked.

The plasma volume changes were neither profound nor prolonged. As indicated in table 1, the maximum deviation in any one experiment was never more than 13 per cent, and in many experiments the observed changes are well within the 5 per cent limit of experimental error generally conceded for the dye method. The nature of the technic, however, is such that systematic error is not likely to affect the essential results, and inasmuch as all of these indicate a change in the same direction, the data may be of some significance even though the values concerned are small. The dilution of the plasma proteins is in fairly good agreement with the dye estimates. Since only two hematocrit measurements were made in each experiment, less reliance can be placed upon these values. As the figures stand, however, they seem to indicate a dilution of the blood of the same order of magnitude as would appear from the dye and protein measurements. Of the twelve animals in which a significant change in plasma volume occurred, three showed recovery to the control level within sixty to eighty minutes. In the same interval, two cats showed an additional concentration beyond the control level, five showed partial recovery, and two showed no recovery at all. A graph of one experiment is given in figure 3.

Table 2 shows the results of ether inhalation by chronic spinal cats. These experiments are of value in the interpretation of the results described above. Neither upon the administration nor upon the withdrawal of ether was any change encountered of the sort that followed the acute transections. In several instances, the induction of anesthesia was accompanied by profound vagal effects, including temporary cardiac asystole and cessation of respiration.

Discussion. The experiments reported here show that in cats under ether anesthesia, low blood pressure following cord transection is usually

accompanied by a small increase in the volume of plasma. The results of the administration of ether to chronic spinal cats make it seem improbable that the plasma volume changes found in the acute procedures are directly related to the state of narcosis. Such a relation is made still less likely, although it is by no means excluded, by Conley's observation (1) that the

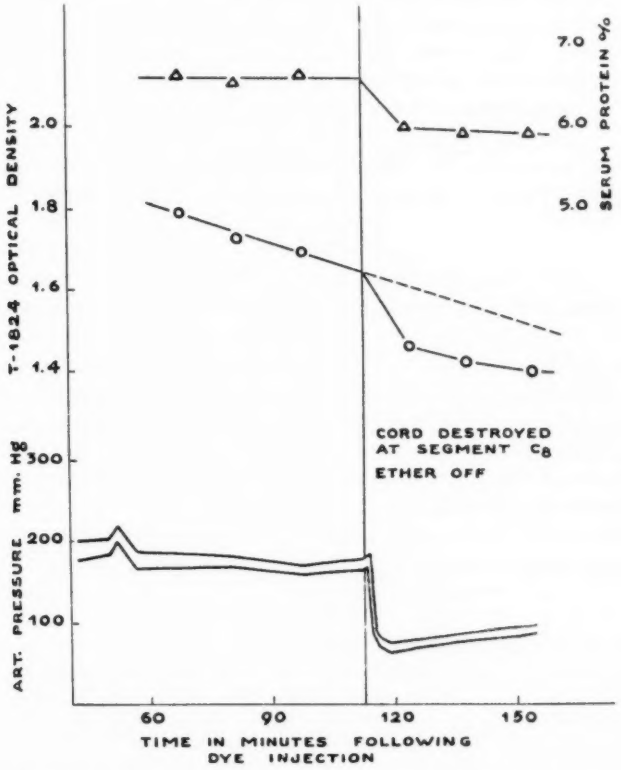


Fig. 3. Graph of experiment 17. Adult male cat with both vagi cut in neck. For photograph of cord lesion, see figure 1.

administration of ether to normal cats is without effect upon the plasma volume. Hamlin and Gregersen (5) found that in cats in which vasoconstrictor pathways are interrupted by total sympathectomy, rather than by cord section as in the present experiments, the volume of the circulating fluid may undergo a large increase.

Various observations indicate that a parallel does not necessarily exist between plasma concentration and arterial pressure reduction. Root and

McAllister (11), for example, showed that inhalation of ether by spinal or sympathectomized dogs results in a fall in blood pressure without any change in plasma volume. In the surgical stage of ether anesthesia, normal dogs exhibited little change in blood pressure, and a consistent reduction of plasma volume (8). The arterial pressure is reported to be reduced by ether in spinal cats as it is in spinal dogs (12), but the data given in table 2 reveal no significant change in plasma volume under these circumstances. The present results, together with those cited above, emphasize the fact that plasma volume changes cannot be predicted with certainty from changes in the arterial pressure alone.

Thanks are due to Dr. Magnus I. Gregersen and to Dr. Walter S. Root for suggesting this problem, and for constant help and encouragement.

SUMMARY

1. A method is described for rapid and relatively bloodless destruction of a segment of the cervical cord of cats by blind puncture and cauterization.

2. Acute destruction of the eighth cervical segment under ether anesthesia was followed by a fall in arterial pressure averaging about 40 per cent, and by an increase in plasma volume averaging 5 to 6 per cent (fig. 3 and table 1).

3. Previous atropinization or bilateral vagotomy did not significantly affect these changes (table 1).

4. Administration of ether to chronic spinal cats resulted in no consistent change in plasma volume (table 2).

REFERENCES

(1) Conley, C. L. This Journal **132:** 796, 1941.
(2) Eppinger, H. and A. Schürmeyer. Klin. Wchnschr. **7:** 777, 1928.
(3) Goldfarb, W., B. Provisor and H. Koster. Arch. Surg. **39:** 429, 1939.
(4) Gregersen, M. I. et al. This Journal **113:** 54, 1935; **125:** 142, 1939.
(5) Hamlin, E. and M. I. Gregersen. This Journal **125:** 713, 1939.
(6) Hermann, H., G. Morin and J. Vial. Arch. internat. de Physiol. **43:** 23, 1936.
(7) Hill, E. F. Brit. J. Surg. **24:** 446, 1937.
(8) McAllister, F. F. This Journal **119:** 363, 1937; **124:** 391, 1938.
(9) Neuhausen, B. S. and D. M. Rioch. J. Biol. Chem. **55:** 353, 1923.
(10) Nowak, S. J. G. Anesth. and Analg. **12:** 232, 1933.
(11) Root, W. S. and F. F. McAllister. This Journal **126:** 613, 1939; **129:** 449, 1940.
(12) Root, W. S. and F. F. McAllister. Unpublished observations.
(13) Sahs, A. L. and J. F. Fulton. J. Neurophysiol. **3:** 258, 1940.

THE SPREAD OF EXCITATION IN TURTLE, DOG, CAT AND MONKEY VENTRICLES[1]

A. SIDNEY HARRIS

From the Department of Physiology, Western Reserve University Medical School, Cleveland, Ohio

Accepted for publication June 28, 1941

The sequence of excitation of the various areas of the ventricular surface of the hearts of experimental animals, mainly dogs and turtles, has been investigated many times and by a variety of methods of leading to electro-registering instruments (18, Chapter I, Methods and Bibliography).

The unipolar method in different hands has yielded results which disagree significantly (15, 19, 1). The validity of the monophasic method employed by H. C. Wiggers (21) has been challenged, since it has been found that an injured area becomes positive upon activity of the surrounding myocardium (8, 17). Differential electrodes used by Clement (3) and by Erfmann (6) have a high electrical resistance, and have been found in our laboratory to yield no deflection in string galvanometer records when arranged to make a small contact and with a string tension that was considered permissible. Coupled to the string galvanometer or cathode ray oscillograph through a suitable amplifier, differential electrodes have recently found considerable use in the study of the relation between local contraction and electrical events (12, 10). The main peak of the differential curve occurs approximately at the moment of contraction and coincides with some point on the steep ascent of negativity in corresponding unipolar curves.

The lack of agreement as to the temporal spread of the excitatory process, and even of the electrical sign indicating its occurrence in unipolar and differential records appears to justify a re-examination of the problem by a different approach. In addition to the experiments on the ventricles of turtles and dogs the study was extended to include the ventricles of cats and monkeys.

RECORDING METHODS. Three large Hindle string galvanometers were arranged to record simultaneously. By means of two mirrors and a shield the central parts of all three light fields were thrown side by side on the face of the camera, approximately perpendicular to it, and without overlapping. Projection distance from each was one meter. A constant speed

[1] This research was supported by a grant from the John and Mary R. Markle Foundation.

rotating wheel with vanes long enough to interrupt the light across the face of the camera provided simultaneous reference lines 40 msec. apart across the whole width of the paper. With the aid of the vertical lines, a binocular magnifier and a 3 msec. scale recorded at the speed of the camera, measurements could be made accurate to within 1 msec. deviation. A large comparator was used on a few records, but it was cumbersome and added nothing to the accuracy of measurement. One galvanometer recorded an electrocardiogram or other reference record, and the others, electrograms from the ventricles. For external surface leads electrodes were desired which could be used with the string galvanometer to record action currents of local origin excluding influences from distant sources to the greatest practicable degree. The high resistance of fluid electrodes and the instability of wicks rendered the differential electrodes of Clement (3) unsuitable for the purpose, though they have the advantage of localized leading. To retain, and possibly improve upon, this property of localization, and eliminate the handicaps mentioned, a new type of electrode assembly appropriately called *riding contiguous bipolar* electrodes was devised. The word *contiguous* is used here in the sense of points that are *very near together but not in contact.* These electrodes consisted of two pieces of 0.035 inch silver wire mounted in a small lucite block (fig. 1). The silver pieces extended about 8 mm. out of the block, and during experiments the ends were close enough together that the overall span of the contact of

Fig. 1. Details of the riding contiguous electrodes. *S*, the silver wires; *J*, jackets; *B*, lucite block; *W*, flexible resilient wires; *L*, supporting lead pipe.

Fig. 2. Records from turtle's heart. Upper electrogram, from contiguous electrodes. Middle electrogram, from unipolar leads. Lower record, R of reference electrocardiogram; 1 and 2, the two slopes of R. Intervals, vertical lines 40 msec.

Fig. 3. Upper electrogram, from external surface of turtle ventricle near apex. Middle electrogram from internal surface immediately opposite external lead. Lower record, reference electrocardiogram. Intervals 40 msec.

Fig. 4. Shapes of unipolar records from different parts of the turtle's ventricle, and the constancy of relation of the onset of the extrinsic portion to the more gradual slope of the reference R. Intervals, tuning fork, 20 msec. Upper electrograms: A, from early area near left base; B, near left mid-ventricle; C, later area just to right of apex. Lower electrograms; R waves from lead II.

Fig. 6. Records from the dog's ventricle. A, B, C and D, upper electrogram, contiguous electrode record; middle, unipolar record, one of the contiguous contacts serving as the stigmatic pole to the unipolar galvanometer; bottom electrogram is from a reference contiguous electrode in the central area. Exploring leads unmoved between A and B, and between C and D.

A and B, from early central area; C and D, from relatively late area near conus. Note small pre-R elevation in C.

E, upper electrogram from point on external surface near base of right ventricle; middle electrogram on internal surface immediately adjacent to external lead. Lower record, reference contiguous electrogram. Intervals, vertical lines 40 msec.

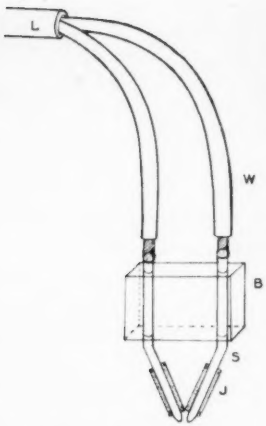


FIG. 1

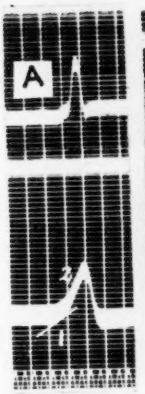


FIG. 2

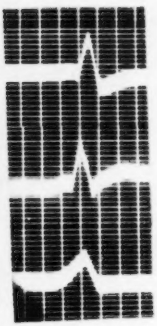


FIG. 3

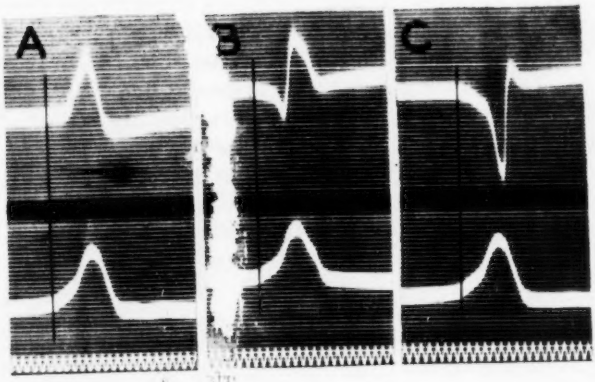


FIG. 4

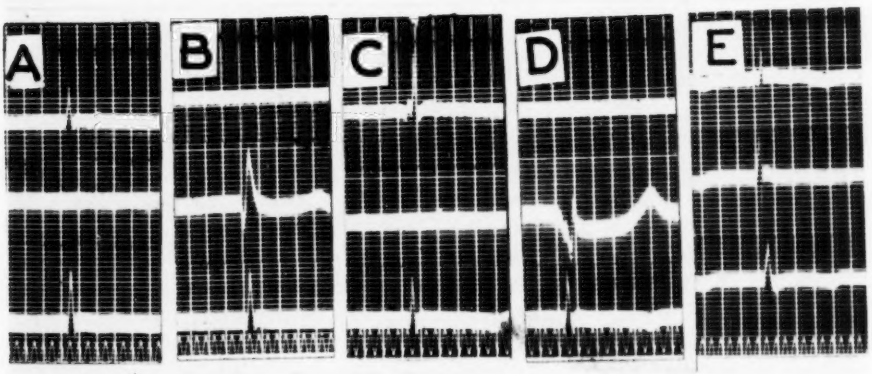


FIG. 6

their tips with the heart was about 1½ mm. The ends should not be sharp, but a long rounded contour helps them maintain their position on a given locus of the surface of the beating heart, and reduces the width of their span. The wires which connect these electrodes with the control box serve also as a resilient suspension for the assembly. This provides for the "riding" property which is essential to the maintenance of a constant contact with the moving ventricle. Wires from discarded Harvard platinum electrodes with all but the last layer of insulation stripped away, and with 1½ to 3 inches extending out of the supporting lead tube were found to permit the electrodes to follow the heart and yet be not too limber. In some experiments with dogs and turtles, records were made from the internal surface of the ventricle by leading with bipolar silver electrodes at the ends of suitably curved insulated tubes inserted via an auricle.

The method of chloriding electrodes is important. Silver electrodes prepared by the Langelaan method (13) were found unsatisfactory for cardiac leads, but a series of experiments yielded a method which produces a high degree of non-polarizability which lasts well under experimental conditions. The silver is cleaned with strips of fine abrasive paper, then immersed to the desired depth in 5 to 10 per cent NaCl and attached to the positive pole of a 3 volt battery. The circuit is completed by a silver or platinum foil electrode attached to the negative pole and immersed. After one minute the connector is moved to the 1½ volt battery post and the process is allowed to continue for 20 minutes longer. After preparation, the electrodes are kept wet with physiological saline.

In use it was found that electrodes which showed no polarization when immersed 3 or 4 mm. in Ringer's solution would polarize perceptibly when removed from the fluid and the ends placed in contact with the heart. In the latter case the area of the electrode-saline interface was very small. When this interface was enlarged by placing a jacket saturated with saline solution around each of the silver pieces the non-polarizable quality was greatly improved. The jackets are practically indispensable also because they maintain a constant level of contact between fluid and electrode, thus preventing potential changes by shifting of fluid levels as the heart moves. Jackets should be removed during cleaning and chloriding of electrodes. A segment from the woven tubular outer layer of a small round shoelace serves well as a jacket.

On theoretical grounds the contiguous bipolar, like the differential electrode, should minimize the recording of potentials of extrinsic origin. Reference to one of the several published diagrams (e.g., 11, fig. 2) showing the distribution of isopotential lines about two oppositely charged points in a plane conductor, or about the active-inactive boundary in tissue makes it clear that closely paired points not in close proximity to the source of potential differences will be practically equally affected. This is not true

if the two leads are far apart, or if one of the pair is remote. Practical tests support these deductions. Contiguous electrode records from the dog's auricles show no ventricular deflection, and ventricular records show no auricular influence. Electrodes on the dog's aorta 2 mm. from the myocardium show no deflection. The freedom from extraneous electrical influences was also demonstrated by applying short D.C. potentials of more than 40 volts through electrodes 1 cm. apart and less than 1 cm. from the leads, without causing excessive movements of the string. In a sample experiment, the recorded potential difference was 0.05 per cent of that applied 1 cm. away. By careful orientation this can be even further reduced. It, therefore, appears safe to conclude that any deflection in the riding contiguous electrode record is the result of electrical activity within a local area of small dimensions.

Rotation of the electrodes, maintaining the same locus as exactly as possible, makes large changes in the spike recorded, but none or very little in the moment of its beginning. The changes are in height, width, and direction. The recorded potential difference (height) varies from maximal to minimal with changes in orientation, but the changes are not entirely predictable. The variation in separation of the limbs ranges (e.g., on the dog's ventricle) from the narrowest that the string can record to a maximum of about 18 msec. These changes cannot be accounted for in terms of conduction along the surface alone, but must involve conduction to the surface fibers. Insufficient information is available concerning the detailed relationships of conduction in the various cardiac tissues to afford a clear explanation at present.

When polarizing currents or brief stimuli evoke idioventricular contractions, much greater changes than those produced by rotation of the electrodes may be seen. The deflections become wider and may assume bizarre shapes. This is especially true at points very near a polarizing electrode, or in discharges resulting from stimuli delivered in late systole, or in multiple trains of discharges from such a stimulus. These changes are due to alterations in rate of conduction and of the direction at which impulses approach the two electrodes. These observations suggest new uses for such electrodes in the study of conduction and its disturbances and alterations.

Turtles. Specimens of *Pseudemys elegans* with carapace width of 5½ to 6 inches were used. Exposure without bleeding was made by trephine opening (3 in. in diameter) in the plastron over the heart region. The leads for the reference electrocardiogram were freshly prepared large silver-silver chloride wires, one of which was embedded in saline saturated cotton just cephalad to the auricles and about 2 cm. to the right of the midline. The other was inserted in the muscle in the left side of the pubic region. Once placed, these electrodes remained undisturbed throughout the experi-

ment. In order to compare the contiguous electrode and unipolar methods of recording and to more surely recognize the local electrical sign in both kinds of records, many experiments were done in which the two kinds of records were made from the same place, one of the contiguous contacts serving also as the active unipolar lead. In such cases records were made from each galvanometer with the other turned off because intercoupling occurred when both of these galvanometers were recorded simultaneously. The electrodes were not moved between these determinations. In records from turtles the sharp onset of the spike in the contiguous electrode record usually coincides with onset of the negative upswing of unipolar electrode records within 5 msec. Ordinarily, the coincidence is closer, frequently exact. Rarely, there are splintered, unusual shaped deflections in unipolar leads that cannot be used.

The relationship of unipolar and bipolar leads to R of the reference electrocardiogram is shown in figure 2. The reference deflection exhibits two slopes marked 1 and 2 (shown also in fig. 4). The examination of many records disclosed that no area on the surface of the turtle ventricle exhibits signs of surface excitation (sharp onset of contiguous electrode record or negative upswing in unipolar record) earlier than about the moment of the bend from the more gradual to the steeper slope of the reference R. The earliest surface records are approximately simultaneous with this bend and excitation at all later areas comes on the steep slope to the peak and beyond the peak. Deflections recorded from the internal surface of the ventricle have been found to precede those from adjacent external areas by 15 to 40 msec., and they occur mainly within the interval occupied by the more gradual slope of R, though from late areas both spikes may occur during the steeper slope (fig. 3).

As shown in figure 2A, the contiguous electrode spike is sometimes preceded by a slight deviation just ahead of the sharp break. This is not synchronous with any feature of the reference electrocardiogram. It is probably of subsurface origin, and does not at all obscure the onset of the spike. In contrast to the deflection recorded by contiguous electrodes which upon correct orientation always display clear spikes, records from unipolar leads on different parts of the ventricle are differently shaped. From the areas that exhibit early surface spikes in contiguous leads the initial deflection (R complex) of the unipolar lead is entirely in the negative direction, the surface excitation being indicated by an abrupt rise of a steep negative slope from one that was low and flat. In areas that show surface excitation latest the deflection is wholly or almost wholly below the base line, movement in the negative direction being chiefly a swift return. Between these there are many gradations corresponding to the relative times of surface excitation (fig. 4). In all cases the positive phase, or the low negative extrinsic phase in regions without a positive phase

begin simultaneously with the more gradual slope of R of the reference electrocardiogram (fig. 4). There is no progression in the onset of the extrinsic phase of unipolar records from the surface of the turtle heart.

RESULTS. The sequence of excitation of surface areas as derived by the methods described is in general agreement with reports that have been made from studies on other species of turtles (14, 16, 12). Figure 5 shows the results of an experiment that may be considered as typical during the spring. The interval between earliest and latest points is 34 msec., and is usually within a few milliseconds of 40. During the winter this interval may be 60 to 70 msec. In late summer it is much less, frequently in the neighborhood of 25. The sequence, however, remains relatively the same. The earliest points are in the left basal and left central areas, and the direction of spread is somewhat diagonally toward the apex and toward the right. The latest point is frequently on the right border no more than halfway from base to apex.

Dogs. Dogs were anesthetized with morphine and barbital sodium. The chest was opened along the sternal midline and the heart suspended in a pericardial cradle. The ventricles of ten dogs were studied. Three pairs of leads could be applied to the ventricle simultaneously. In some experiments they were all contiguous sets, one for a constant reference record and two for punctate measurements.

RESULTS. Records from contiguous electrodes consist of an initial complex and a T wave (fig. 6). The initial complex has a prominent sharp spike deflection R, and from some areas, one or more small (pre-R) deflections, shown in figure 6, C. The first pre-R event may precede the main spike by any interval from 0 to 20 milliseconds. In some records it is merely a bevel of 1 to 3 msec. leading to the sharply breaking upturn of R, and in others no preceding event is detectable. The interval between pre-R and R is smallest in the thin-walled central and trabeculated areas of the right ventricle. Records from various points on the right ventricle made with internal and external leads opposite each other and separated only by the thickness of the myocardium show that the deflection from the internal lead (fig. 6, E) always preceded the external deflection. In the thin central portion where early surface responses occur, the interval of internal precedence ranges from 1 to 5 msec., while over the rest of the right ventricle, sampling all parts except the conus, the difference was 9 to 12 msec. The range of interpunctate intervals on the endocardial surface is small. The maximum in the right ventricle is about 6 or 7 msec. The internal spike appears to have a close association with the pre-R surface events, but they are not identical. When the pre-R elevations were multiple the internal spike occurred during one of them; when single, they appeared simultaneously, or the internal spike fell within the duration of the pre-R deflection. The internal spike usually was narrow and

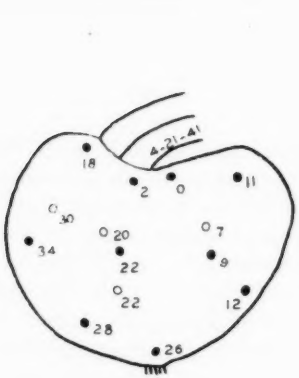


FIG. 5

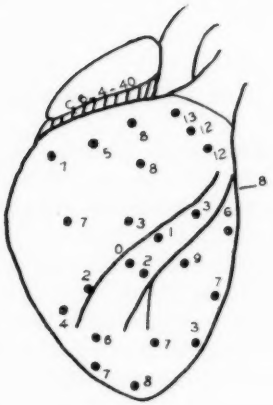


FIG. 8

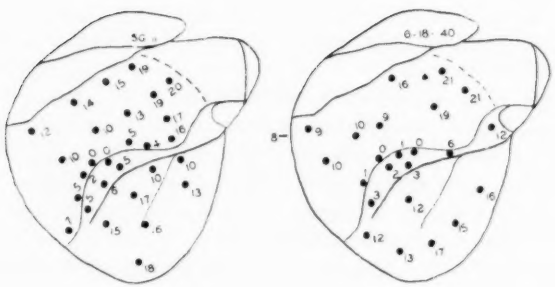


FIG. 7

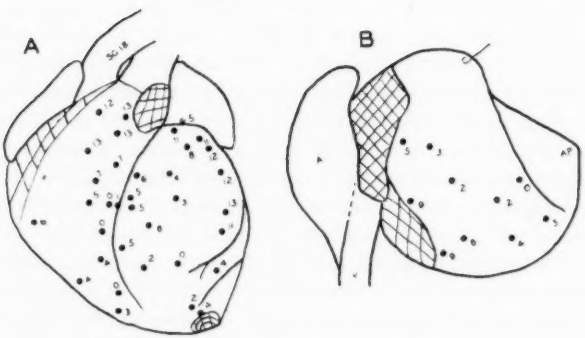


FIG. 9

Fig. 5. Surface excitation of the turtle heart. Solid dots, ventral surface; circles dorsal surface. Figures show intervals in milliseconds after excitation of the earliest point recorded. Spring experiment: April 21st.

Fig. 7. Surface excitation of the dog's heart. Two experiments. Figures, milliseconds after earliest point recorded.

Fig. 8. Surface excitation of the cat's heart. Sample experiment. Figures, milliseconds after earliest point recorded.

Fig. 9. Surface excitation of monkey's heart. A, ventral surface, and extending a considerable distance on the dorsum of the left ventricle. B, heart drawn far over to expose the side and dorsal aspects of the right ventricle. Figures, milliseconds after earliest point recorded. Cross-hatched areas are fat pads.

sharp. The pre-R deflections probably are not records of action currents in the subendocardial conducting tissues themselves, but they are associated with conduction beneath the external surface of the ventricle.

The relations of bipolar and unipolar leads to a standard bipolar lead from another area are shown by comparison of A, B and C, D of figure 6. Unipolar records contain a positive phase followed by a returning upswing which continues beyond the base line as a negative wave (fig. 6 B). The relative prominence of these two vary with the time of excitation of the areas, much as in the case of the turtle ventricle (cf. fig. 6, B and D). These measurements were made by contiguous and unipolar leads from the same placement of electrodes, one of the contiguous points serving as the stigmatic pole of the unipolar pair. As in the turtle experiments, the galvanometer connected to the unipolar leads was turned off (infinite resistance), while the contiguous electrode record was being made and vice versa, but the electrodes were not moved between records. The deflections in all leads are much more consistently sharp and unequivocal from mammalian hearts than from the turtle. It is exceedingly rare that one has cause to question the instant of onset of the contiguous electrode spike within 1 msec. and the unipolar upstroke in the negative direction is usually free of complication. Occasionally there is an angulation of five to ten milliseconds' duration, ending in a sharp bend upward. In all cases the onset of the contiguous spike and the sharply upbreaking point of the unipolar deflection coincide most closely, rarely varying from each other by more than 2 msec. Neither event bears any significant relation to the onset of the positive phase of the unipolar record.

Figure 7 shows the relative times of surface excitation at points on the ventral aspect of the right and left ventricles in two dog experiments which may be considered typical. As previously reported by others (15, 19), the earliest points are found in the central area on the trabeculated region of the right ventricle and near mid-septum. The process arrives at other areas of the right and left ventricles later, the conus latest of all, though in some hearts it is not excited much later than some points on the left ventricle. A few records made from the vortex were found to be quite early, 4 to 7 msec. after the earliest point. In no experiment was the span between earliest and latest points on the ventricular surfaces more than 22 msec., with a variation from 18 to 22. These figures considered in conjunction with those derived by internal leading and internal-external pairs tend to confirm the deductions of Lewis and Rothschild (15) that the impulse arrives at all parts of the endocardium within a very brief interval and from there proceeds through the myocardium to the surface much more slowly.

Cats. The hearts of eight cats were studied. These animals were anes-

thetized with dial[2] and the thoracic contents exposed by midsternal sagittal opening in a manner generally similar to that used in the dog. The ventricles were explored with the contiguous electrodes and the relative excitation times plotted. As in the dog ventricles, there is a degree of orderly sequence, but with some variations in absolute values found for corresponding points in different hearts. Figure 8 shows the results of one experiment which may be considered as a fair sample. Like the dog ventricles, the earliest area is near mid-septum and in the immediately neighboring region of the right ventricle. The relationships in other regions resemble those of the dog heart also, but the values are smaller, generally one-half to two-thirds of the figure for the corresponding region of the dog ventricle. In some cat experiments, one difference was noted. In regions along the margin of the left ventricle ordinarily exhibiting an action current 6 to 8 msec. after the earliest there would be an occasional reading that was very early, only 1 or 2 msec. after the earliest. The meaning of this has not been learned. Erfmann's (6) measurements on cats' hearts with differential electrodes were chiefly from the base and apex. Some of his base-apex intervals are greater than any found in these experiments. Surface contiguous electrograms from cats and monkeys show pre-R elevations qualitatively like those in dog records.

Monkeys. Two hearts of Macacus rhesus monkeys were mapped, many points being recorded from both dorsal and ventral surfaces. The results of one of these experiments are shown in figure 9. The pattern of surface excitation appears similar to those of the dog and cat with respect to certain large features, but there are also differences which may be significant. Similarities are points of earliest excitation in the central region of the right ventricle near the septum, and late areas on the conus region of the right ventricle and at the more basal and dorsal portions of the left, the latter being excited relatively later in the monkey's heart. The most striking difference is in the irregular and widespread area which receives excitation early, as shown in figure 9A. The whole right ventricle, except the conus region and a basal rim band extending far around on the dorsal aspect is excited within 5 msec. In the experiment illustrated in figure 9A, the area of early excitation also includes the apex and right half of the left ventricle, though there are points immediately left of the septum which are later than apical and mid-ventral portions of the left ventricle. Measurements on the two hearts agreed closely except on the latter two areas, the apex and mid-ventricle on the heart not illustrated showing values of 6 and 7 msec. after the earliest. The breadths of span from earliest to latest points were 13 and 14 msec. in the two hearts.

[2] The dial used in the experiments on cats and monkeys was generously contributed by the Ciba Company, Inc.

Discussion. A consideration of the fundamental nature of excitation or of action potentials is not the purpose of this presentation, but in order to measure the temporal spacing of events one must be able to recognize their signs. It has been seen that the onset of the portion (positive or negative) of the unipolar complex preceding the quick upstroke led from any part of the ventricle coincides in time with the beginning of the more gradual slope of R of the electrocardiogram which is temporally associated with excitation and conduction in tissues within the turtle ventricle (perhaps the auricular funnel tissue). Therefore, this part of the unipolar record can be dismissed as being no part of the electrical manifestation of local surface excitation. The close agreement between the onset of the spike in the contiguous electrode record and the steep upstroke of the unipolar record supports strongly the old idea that the sharp movement in the negative direction is the unipolar manifestation of an action current in the local surface area. This finding agrees not only with the great mass of observations from complex tissues but also those of recent experiments in which excitation has been studied under conditions of minimal anatomical complication. When electrodes were placed opposite each other, one on the inside and the other on the outside of the membrane of the squid giant axon the action potential with reference to the outside lead consists of a negative spike and an after-potential (5). It has recently been demonstrated that the large decrease in electrical impedance (depolarization?) across the membrane of the squid giant axon and of Nitella which accompanies excitation by a propagated impulse occurs during the rising phase (negative) of the monophasic action potential. The part of the action potential preceding the local membrane changes is tentatively interpreted as a passive fall of potential affecting the electrode ahead of the approaching partial short circuit (4). If this explanation is correct, action potential leads sufficiently near together should tend to eliminate the preceding fraction and approach the limit of zero interval between the onset of the action potential and the fall of impedance. Bishop (2) has referred to studies of electrical phenomena occurring on excitation of plant and animal cells, citing them as evidence that the process of excitation is general.

The variations in the interval found between excitation of earliest and latest external surface points on the turtle ventricle agree quite well with those of Lewis (14) if both summer and winter experiments are included.

The results of measurements on the dog's ventricles agree quite closely with some of those of Lewis and Rothschild (15) and of C. J. Wiggers (19). The same general sequential pattern exists and frequently there is close quantitative agreement. The main difference found is in the magnitude of the variations reported in some of their experiments. Instead of an interval of 18 to 22 msec. between earliest and latest points in different

experiments and a maximal variation of 4 or 5 msec. between corresponding points, they report wider variations. In the experiments of Lewis and Rothschild the interval between earliest and latest points was nearly always greater than 20 msec. and frequently approached 30. C. J. Wiggers' variations were from less than 20 msec. to more than 30. Doctor Wiggers has expressed the opinion that the stitching of the wick lead to the myocardium, though done in many experiments with an oiled horse-hair, may have affected the records.

The experiments with cats may offer a clue to some of the variations seen in experiments on dogs. The cats' hearts used were much smaller than those of the dogs, but the excitation pattern was quite similar. The differences are quantitative and appear to obey a fairly constant proportionality throughout the various areas. This suggests that the differences in intervals noted between cats' hearts and dogs' hearts may be a function of size. Our dogs were of fairly uniform weight, 9 to 12 kgm., and therefore do not give a conclusive answer. It appears possible, too, that some of the largest figures of others for interpunctate intervals were derived from very large hearts.

The results reported by Abramson and Jochim (1) were obtained with the exposed thoracic contents covered by a warm moist chamber. The figures which they chose as the true ones showed maximal interpunctate intervals on the ventral surface of the dog's ventricle of no more than 8 to 9 msec. They attribute the greater differences in the results of Lewis to surface cooling and drying of the exposed heart. The factor of drying has been prevented in our experiments by meticulous care to keep the ventricle moist by an automatic dropping system. To test the "cooling hypothesis" the surface temperature of a ventricle which had been used with the chest open for six and one-half hours and which had been fibrillated and defibrillated a number of times was measured with a Tycos dermatherm with the stigmatic junctions of the thermopile covered with cellophane. The rectal temperature was 37.2°C., the room temperature 26, and that of the ventricular surface, 37.0. This is in confirmation of a series of measurements by Wiggers and Wegria (20) who found that the temperature of the cardiac surface in open-chested preparations closely approximates that of the blood. The results of Abramson and Jochim need a different explanation and confirmation.

The experiments of Erfmann (6) with differential electrodes are sometimes cited as evidence that the various areas of the dog's ventricle are excited simultaneously. But Erfmann's protocols show interpunctate intervals which might be considered large, e.g., in experiment IX, base-apex differences range from 6 to 18 msec. The largest of these rank among the highest of comparable values reported by Lewis and Rothschild or C. J. Wiggers. The experiments of H. C. Wiggers (21) using the mono-

phasic method of recording showed exactly simultaneous moments of onset of the monophasic curve over large ventricular areas, and then other series whose values differ from the first group, but are simultaneous within each areal grouping. Such groupings appear to be in accord with the statement of Eyster, Meek, Goldberg and Bartsch (9) that the monophasic method measures the moment of onset of activity in areas adjacent to the injury rather than in loci to which the "active" electrode is applied.

Eyster, Meek and Gilson (7) measured the sequence of occurrence of differential peaks from loci distributed over the dog's ventricle. The information in their brief report is consistent with the measurements of onsets of contiguous electrode spikes or unipolar negativity, but only gross deviations would be subject to detection. In view of the findings of Cole and Curtis, it is possible that the peak of the differential curve may offer a fair approximation to the moment of impedance fall. However, in consideration of the fact that rotation of contiguous electrodes changes the width of the spike and the temporal distance of the peak from the onset, the onset may be expected to bear a more constant relation to the moment of the first local excitation change under the earlier of the lead points than does the peak.

SUMMARY

Methods of electrographic leading have been considered and a new type of simple leading electrode assembly, riding contiguous electrodes, described. Evidences are offered to show that cardiac action currents recorded by them are local in origin.

Two slopes are described in the electrocardiographic R wave of the turtle. The earlier, more gradual one, occurs during the time that local spikes can be recorded only from inside the ventricle, and the steep slope and a short time beyond the peak occupy the interval during which signs of surface excitation are recorded. The beginning of the extrinsic part of the initial unipolar complex from all areas is simultaneous with the beginning of the more gradual slope of R.

On both the ventral and dorsal surfaces of the turtle ventricle surface excitation occurs earliest in the left-basal area and progressively later in areas toward the right of the apex. The interval between earliest and latest surface points may vary from 25 to 70 msec., varying with the season.

From the dog's ventricles the onset of contiguous spikes was found to coincide with the upstroke in the unipolar record, as from turtle hearts, but the records are sharper. Small pre-R deflections ahead of the contiguous electrode spikes are described, and they were found to be associated with the spike from the internal surface in an inconstant manner. The pre-R deflection is ascribed to conduction in fibers below the surface.

The sequence of surface excitation in the dog's ventricles was found to

confirm generally the findings of Lewis and Rothschild and C. J. Wiggers, but the maximal intervals between areas was less than many of theirs. Factors which might account for this are discussed. Comparative measurements from other investigators are considered. Leads from the internal surface of the dog's right ventricle indicate that the whole endocardial surface is activated within a very brief interval and the excitation passes through the wall much more slowly.

The spread of excitation over cats' ventricles was found to follow the same general pattern as in the dog, but the intervals are only about two-thirds as great or less. The difference may be accounted for by relative sizes.

The pattern of excitation of the ventricles of Macacus rhesus monkeys differs somewhat from that of dogs and cats, but the greatest intervals between surface points are approximately equal in cat and monkey hearts of similar sizes.

Recent fundamental experiments on the process of excitation are mentioned, and from them is suggested the possibility that the onset of the sharp contiguous electrode spike approximates the instant when the large fall of electrical impedance, and hence perhaps depolarization, occurs.

REFERENCES

(1) Abramson, D. I. and K. Jochim. This Journal **120:** 635, 1937.
(2) Bishop, G. H. Ann. Rev. Physiol. **3:** 1, 1941.
(3) Clement, E. Ztschr. f. Biol. **58:** 110, 1912.
(4) Cole, K. S. and H. J. Curtis. J. Gen. Physiol. **22:** 37, 649, 1939.
(5) Curtis, H. J. and K. S. Cole. This Journal **129:** P342, 1940.
(6) Erfmann, W. Ztschr. f. Biol. **61:** 155, 1913.
(7) Eyster, J. A. E., W. J. Meek and W. E. Gilson. This Journal **123:** 60, 1938.
(8) Eyster, J. A. E., W. J. Meek, H. Goldberg and W. E. Gilson. This Journal **124:** 717, 1938.
(9) Eyster, J. A. E., W. J. Meek, H. Goldberg and H. L. Bartsch. This Journal **126:** P488, 1939.
(10) Eyster, J. A, E., W. J. Meek and H. Goldberg. This Journal **131:** 760, 1941.
(11) Gilson, A. S. and G. H. Bishop. This Journal **118:** 743, 1937.
(12) Goldberg, H. and J. A. E. Eyster. This Journal **128:** 390, 1940.
(13) Langelaan, J. W. Arch. néerl. de Physiol. **14:** 592, 1929.
(14) Lewis, T. Phil. Trans. Roy. Soc. B **207:** 221, 1916.
(15) Lewis, T. and M. A. Rothschild. Phil. Trans. Roy. Soc. B **206:** 181, 1915.
(16) Meek, W. J. and J. A. E. Eyster. This Journal **31:** 31, 1912.
(17) Sugarman, H., L. N. Katz, A. Sanders and K. Jochim. This Journal **130:** 130, 1940.
(18) Wiggers, C. J. Circulation in health and disease. 2nd ed., 1923.
(19) Wiggers, C. J. This Journal **80:** 1, 1927.
(20) Wiggers, C. J. and R. Wégria. This Journal **131:** 296, 1940.
(21) Wiggers, H. C. This Journal **118:** 333, 1937.

THE REMOVAL OF DIODRAST FROM BLOOD BY THE DOG'S EXPLANTED KIDNEY

A. C. CORCORAN, H. W. SMITH AND IRVINE H. PAGE

From the Lilly Laboratory for Clinical Research, Indianapolis City Hospital, Indianapolis, Indiana, and the Department of Physiology, New York University College of Medicine, New York City

Accepted for publication June 27, 1941

The clearance of diodrast from plasma in human beings is believed to be approximately equal to the rate of flow of blood plasma through excretory renal tissues (Smith, Goldring and Chasis, 1938). Indirect evidence suggests that the removal of diodrast from renal blood in human beings is nearly complete (Smith, 1940).

The present report is concerned with direct measurements of the removal of diodrast from blood by the subcutaneously explanted kidney of dogs.

METHODS. One or both kidneys were subcutaneously explanted by the method of Page and Corcoran (1940), the non-explanted kidney being removed at a subsequent operation. Observations on diodrast excretion were not begun until three or more months from the time of last operation.

Diodrast, phenol red and inulin in 0.9 per cent NaCl solution were infused intravenously at about 1 cc. per minute, in concentrations designed to maintain phenol red (P) and inulin (I) at plasma levels of 0.7 and 70 mgm. per 100 cc., respectively, while the diodrast content was varied according to the plasma concentration of diodrast desired in the experiment. Administration of the infusion was preceded by a smaller priming dose of a more concentrated solution. Observations were usually begun 20 to 30 minutes after the priming dose had been given. The preparation of solutions, collection of urine and blood, determinations of phenol red and inulin concentration, calculation of renal clearances, extraction ratios and renal plasma flow have been described by Corcoran and Page (1939).

For brevity the following abbreviations are used: D, diodrast iodine concentration; PW, plasma water; CW, cell water; C_D, diodrast clearance in cubic centimeters of plasma per square meter body surface per minute; E, renal extraction ratio, which is calculated as (A-V)/A, where A and V are, respectively, the simultaneous arterial (or jugular) plasma concentration, and V is the renal venous plasma concentration, of phenol red, inulin or diodrast. Renal plasma flow (RPF) in cubic centimeters per square meter per minute was calculated and averaged from the plasma clearances

and extraction ratios of phenol red and inulin by the formula C/E. Plasma water content (P_w) was assumed to be 93 per cent and red blood cell water content (C_w) 74 per cent.

The diodrast clearance in human beings is depressed at high plasma concentrations because of overloading of the tubular excretory process, which has a limiting maximal rate (Smith *et al.*, 1938). A similar limitation in tubular excretion is present in the dog, as has been demonstrated for phenol red by Shannon (1935) and for diodrast and other substances by Smith (unpublished observations). Since increasing either RPF or D increases the load of diodrast carried to the tubules, it is appropriate, in comparing various observations, to consider this "load" (RPF × D) rather than plasma concentration alone.

Diodrast analysis. Samples of urine and plasma collected in Indianapolis were sealed in glass ampoules and mailed to New York for the determination of diodrast content by the method described by Smith, Goldring and Chasis (1938). The adequacy of this method for recovery of diodrast iodine from plasma at concentrations lower than those met in the analyses of renal venous blood was demonstrated by a substantial series of recoveries. Inorganic iodine blanks (0.02 to 0.04 mgm. per 100 cc.) were in some instances observed in the analysis of samples of plasma obtained before diodrast was given, and in these instances a correction was made by subtracting the bland from both arterial and renal venous diodrast concentration. This correction was made on the assumption that this iodine is excreted with low and negligible clearance. The method of White and Rolf (1940) was used in analyses made in most of the specimens from dogs with bilaterally explanted kidneys. Analyses of red blood cell D were obtained from the difference between whole blood and plasma values.

Results. *Distribution of diodrast in plasma and red cells.* The ratio D_{CW}/D_{PW} in arterial (or jugular venous) plasma of dogs averages 0.49 and ranges from 0.33 to 0.61 in 15 observations made during constant or very slowly changing D. Ratios ranging from 0.12 to 0.67 were encountered in observations made during rapid shifts of D. Low ratios are associated with rising D and *vice versa*, and the ratio is restored towards the mean as D is maintained for 10 to 20 minutes. The value of the ratio is independent of the absolute value of D.

The ratio D_{CW}/D_{PW} in renal venous blood varies from 0.31 to 2.33 and is highest at high arterial D.

The ratio $\frac{D_{CW} \text{ renal vein}}{D_{CW} \text{ renal artery}}$ averages 0.72 in 11 observations at renal venous D_{PW} of 0.15 to 1.4 mgm. per cent, and varies from 0.55 to 1.28. In 6 observations at higher renal venous D_{PW} (2.0 to 3.3 mgm. per 100 cc.) the mean is 0.79.

Extraction ratio of diodrast. Plasma. The mean plasma extraction ratio of diodrast (E_p) from plasma in uninephrectomized dogs with single ex-

planted kidneys is 0.84, with extremes of 0.79 to 0.96 in 19 observations at renal loads of from 0.5 to 4.9 mgm. per square meter per minute. E_p decreases at higher plasma loads (fig. 1), no doubt because of encroachment on the maximal rate of tubular excretion. In dogs with bilaterally explanted kidneys, E_p averages 0.83 (9 observations) and ranged from 0.73 to 0.89 at renal loads of from 0.5 to 10 mgm. of iodine per square meter per minute. A few anomalously low values, possibly due to contamination of the renal venous sample with arterial blood or with urine, or to the opening of arterio-venous shunts in the kidney, are not included in this report.

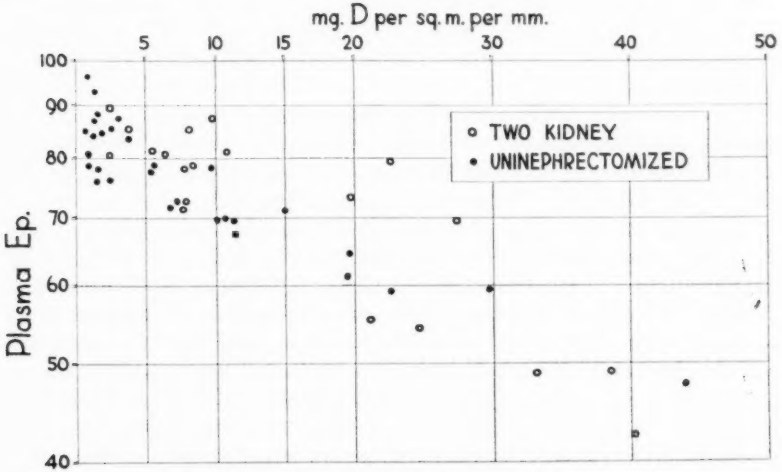


Fig. 1. The relation of renal diodrast extraction percentage to apparent renal plasma load. Ordinate: renal plasma load of D. in milligram per square meter of body surface per minute (RPF_{XD_p}). Abscissa: diodrast extraction percentage $\frac{(A-V)}{A}100$.

Red blood cells. Diodrast extraction from red cells (E_c) in dogs with bilaterally explanted kidneys averages 0.203 at renal plasma loads of from from 0.5 to 9.9 mgm. of iodine per square meter per minute and ranges from 0.043 to 0.514 (10 observations). At renal loads of from 10 to 40 mgm. of iodine per square meter per minute, the mean of 7 observations is 0.215. Three instances of apparent addition of diodrast to red cells in the kidney were not included in the calculation of those means.

Whole blood. Diodrast extraction from whole blood (E_b) at renal loads of from 0.5 to 10 mgm. D per square meter per minute averages 0.707 in 9 observations in a dog with bilaterally explanted kidneys, and ranged from 0.64 to 0.82.

Diodrast clearance and renal blood flow. In those experiments at low

loads (0.5 to 5 mgm. of iodine per square meter per minute) in uninephrectomized dogs, the mean ratio of plasma C_D to RPF is 0.87 (25 observations), the apparent renal plasma flow, as calculated from C_D/E_p is 1.06 RPF. Two experiments (7 observations) in dogs with bilaterally explanted kidneys yield similar values. The ratio $\frac{\text{whole blood } C_D/E_b}{\text{renal blood flow}}$ was 0.97 in these.

Discussion. *Distribution of diodrast in cells and plasma.* White (1940) has confirmed the observations of Smith, Goldring and Chasis (1938) that diodrast enters red blood cells *in vitro* very slowly, but has found that penetration *in vivo* occurs rapidly. Apparently equilibrium is reached before 20 minutes. The distribution ratio (D_{CW}/D_{PW}) found by White (0.58) is in fair agreement with our average of 0.49. The reason why diodrast is not distributed uniformly per unit of plasma and cell water is unknown.

The variability of the ratio (D_{CW}/D_{PW}) in renal venous blood and its general proportionality to the diodrast content of arterial blood is presumably due to failure of establishment of cell/plasma equilibrium in the short time of a single renal passage (White, 1940).

The diodrast content of renal venous cell water is nearly always lower than that of arterial cells. The mean ratio of these values of 0.72 found by us at low plasma levels agrees with the value of 0.77 found by White (1940).

Renal extraction of diodrast. The mean extraction of diodrast from plasma in dogs at low renal loads is apparently unaffected by uninephrectomy, although the lower tubular mass of uninephrectomized dogs causes depression of extraction at lower renal loads than in dogs in which both kidneys are present. Our mean value (0.84) is somewhat higher than that found by White (1940) in dogs with single explanted kidneys (0.74). This difference is possibly due to the fact in White's observations the mean included all data obtained at plasma D of less than 13 mgm. per 100 cc., whereas our average is based on data obtained at relatively low loads.

The mean extraction from red blood cells in our observations agrees with the value of 0.23 found by White (1940). Apparent addition of diodrast from plasma to the red blood cells during renal passage was noted in three instances. The significance of this observation is not clear.

The extraction from whole blood in our observations in dogs with bilaterally explanted kidneys is 0.707 in contrast to the value of 0.56 found by White (1940). By our average E_p of 0.84, mean E_c of 0.20 and arterial (D_{CW}/D_{PW}) of 0.48, the calculated E_b should be 0.69. Similarly, in the data reported by White (1940), the mean E_p of 0.74, E_c of 0.23 and arterial (D_{CW}/D_{PW}) of 0.57 give a calculated E_b of 0.59, as compared with the observed value of 0.57. Thus the observed E_b in both cases agree with the values calculated from independent data. This agreement suggests that the differences in E_b between White's observations and those reported here are systematic.

Diodrast clearance and renal blood flow. The contribution of a fraction of the red blood cell diodrast to the urinary excretion renders the measurement of true renal plasma flow uncertain in the absence of analysis of renal venous blood, as White (1940) has pointed out. In the present observations RPF = 0.943 C_D/E_p, as compared with 0.89 C_D/E_p in White and Heinbecker's observations (1940), deviation from unity representing the contribution of diodrast from the cells. Balancing this contribution from the red cells (6 per cent) is the incompleteness of plasma E_p (14 per cent). Diodrast plasma clearance is therefore less than true renal plasma flow and the ratio RPF/CD averages 0.87. True renal plasma flow is therefore 1.15 CD while the value obtained by White and Heinbecker (1940) was 1.2 CD.

We may add the cautionary note that because of occasional low extraction ratios observed by White and Heinbecker and ourselves, and because the explanted kidney is perhaps subject to abnormal stresses, especially on the renal vein, and frequently shows some thickening by the capsule and scarring, we are not fully convinced that the values for the extraction ratio here reported represent the normal average value in the kidney *in situ*.

SUMMARY

Diodrast intravenously infused in dogs is unequally distributed between the plasma and red blood cells, the distribution ratio between cell and plasma water being approximately 0.50.

At low renal loads, the extraction of diodrast from arterial plasma during its passage through the explanted kidneys of dogs averaged 0.84. Diodrast extraction was not affected by uninephrectomy.

Renal extraction of diodrast from arterial red blood cells averaged 0.20.

The removal of diodrast from arterial blood by the dog's explanted kidney is such that at low renal loads the ratio $\frac{\text{plasma diodrast clearance}}{\text{renal plasma flow}}$ is approximately 0.87.

REFERENCES

Corcoran, A. C. and I. H. Page. This Journal **126:** 354, 1939.

Goldring, W. H., H. A. Chasis, H. Ranges and H. W. Smith. J. Clin. Investigation **19:** 739, 1940.

Page, I. H. and A. C. Corcoran. Surgery **7:** 389, 1940.

Smith, H. W., W. H. Goldring and H. A. Chasis. J. Clin. Investigation **17:** 263, 1938.

Smith, H. W. The Harvey Lectures, 1940.

Smith, W. W. and H. W. Smith. J. Biol. Chem. **121:** 107, 1938.

Van Slyke, D. D., A. Hiller and B. F. Miller. This Journal **113:** 611, 1935.

White, H. L. This Journal **130:** 454, 1940.

White, H. L. and D. Rolf. Proc. Soc. Exper. Biol. and Med. **43:** 1, 1940.

White, H. L. and P. Heinbecker. This Journal **130:** 464, 1940.

Shannon, J. A. This Journal **113:** 602, 1935.

THE TRANSFER OF RADIOACTIVE SODIUM ACROSS THE PLACENTA OF THE GOAT

HERBERT A. POHL, LOUIS B. FLEXNER AND ALFRED GELLHORN

From the Department of Embryology, Carnegie Institution of Washington, Baltimore, Md.

Accepted for publication July 7, 1941

This study is the fourth of a series on the comparative physiology of placental transfer. It follows observations (1, 2, 3) on placental transfer of radioactive sodium across the hemochorial placenta (maternal and fetal circulations separated by chorionic epithelium and endothelium of fetal blood vessels) and the endotheliochorial placenta (maternal and fetal circulations separated by endothelium of maternal blood vessels, chorionic epithelium and mesenchyme, and endothelium of fetal blood vessels). The placenta of the goat is syndesmochorial, i.e., the maternal and fetal circulations are separated in order by endothelium of maternal blood vessels, maternal connective tissue, chorionic epithelium, fetal connective tissue and endothelium of fetal blood vessels. As in the earlier reports, this study has as its purposes an evaluation of transfer rate across a unit weight of placenta at various stages of pregnancy; a correlation of the transfer rate and morphology of the placenta; and a comparison of the relative growth rate of the fetus and the rate at which sodium is supplied to a unit weight of fetus at different periods of gestation.

EXPERIMENTAL PROCEDURE. The general experimental procedure has previously been fully presented (1, 2). Radioactive sodium (Na^{24}), present as the chloride, was prepared by use of the Harvard cyclotron. Approximately 30 microcuries were taken for injection into an ear vein of a pregnant animal. The radioactivities of fetal ash and maternal plasma were measured with a pressure ionization chamber-string electrometer circuit (1). Ether was given to the animals before delivery of the fetuses by Caesarean section. Pregnant animals were obtained from a local herd of domestic goats and, except where noted, the gestation age was known.

The units of radioactivity which have been used are those previously given (1). The term "corrected" placed after values for Na^{24} means that these values have been corrected to a concentration of one beta-particle per second per cubic centimeter of maternal plasma.

RESULTS. With the guinea pig (1), cat (2) and rat (3), the first experimental step was to establish the curve describing equilibration of the fetus

with the Na^{24} in the maternal plasma. This permitted the removal of the fetus at a time when its concentration of Na^{24}, derived from the maternal plasma, was increasing in a linear manner. In view of information gained from previous experience and the limited amount of experimental material, it was considered inadvisable to establish the equilibrium curve for the goat. Instead, the volume of distribution of Na^{24} in the goat fetus near term was assumed to be like that of the guinea pig. This value in the goat fetus at this period is probably about 30 per cent of the body weight. The transfer rate of Na^{24} is of such magnitude (fig. 4) that at the end of two hours the fetus has approximately one-sixth the concentration of Na^{24} anticipated at equilibrium. This deduction was verified in one young fetus by making sodium analyses on the maternal plasma and fetal ash, from these analyses calculating the apparent volume of distribution of Na per unit body weight, and then comparing the rate of transfer of Na^{24} to the equilibrium value established in this way. Previous experience has shown that the apparent rate of exchange between maternal plasma and fetus is linear up to and somewhat beyond one-sixth of the concentration found at equilibrium in the fetus. The routine procedure for measuring placental transfer in the goat has consequently been to remove the fetuses by Caesarean section at a known interval of about two hours after intravenous injection of Na^{24} into the mother.

The change of placental weight with change of fetal weight is given in figure 1. The lack of correlation between these two quantities is notable. The placental weight as noted refers to the combined weights of the fetal cotyledons and does not include the intercotyledonary chorion. The total Na^{24} transferred to the fetus per unit time, as this varies with change of fetal weight or gestation age, is shown in figure 2. The data of figures 3 and 4 have been derived from those of figures 1 and 2.

Figure 3 shows the changes in rate of transfer across a unit weight of placenta from about the tenth week of pregnancy until term. There is a three or fourfold increase from about the tenth to the nineteenth and twentieth weeks of gestation and then a decrease to term.

Figure 4 gives in per cent the daily relative weight increase of the fetal goat as this varies with fetal weight. The daily per cent weight increase has been calculated from that part of the data of figure 2 which relates fetal weight to known gestation age; the method of calculation has previously been given (1). Figure 4 also gives the transfer rate of Na^{24} per gram fetus per hour as this changes with fetal weight. The two curves are similar. The ratio of their ordinates at corresponding fetal weights is as follows: at 100 grams, 1.5; 200 grams, 1.4; 500 grams, 1.9; 1000 grams, 1.6; 2000 grams, 1.5.

The essential data are lacking for calculation of the safety factor for sodium (ratio of rate of supply of sodium to the fetus to rate of accretion

of sodium by the fetus) at all stages of gestation. If it be estimated, however, that the volume available to Na is 30 per cent of the body weight at

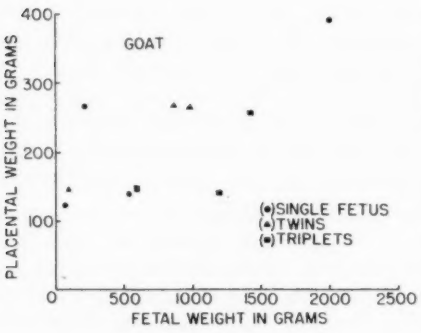


Fig. 1

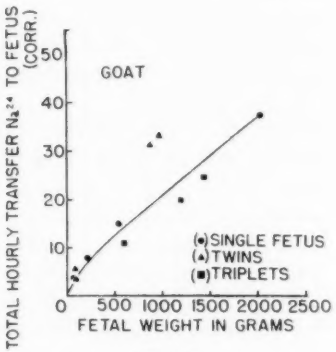


Fig. 2

Fig. 1. Variation of placental weight with variation of fetal weight. The gestation ages of the fetuses are given in figure 3.

Fig. 2. Variation of total hourly transfer of radiosodium to fetus with variation of fetal weight. The measured radioactivity of the fetal ash has in each instance been corrected to a concentration of one beta-particle per second per cubic centimeter of maternal plasma.

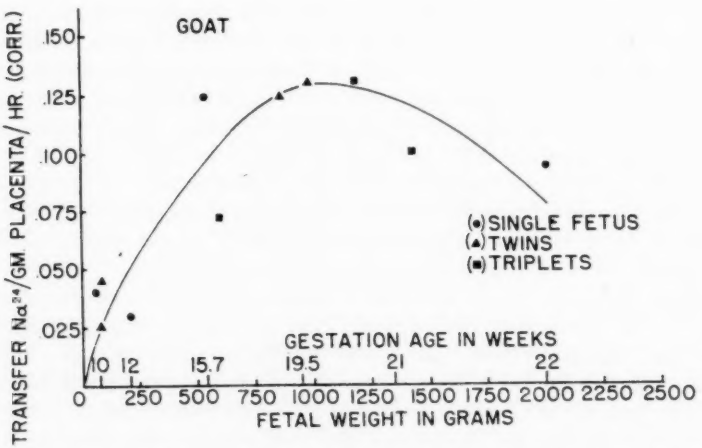


Fig. 3. Variation of transfer rate of Na^{24} per unit weight of placenta with variation of fetal weight or gestation age. The gestation age of the triplets and of the twins weighing 870 and 990 grams was unknown.

or near term, the safety factor can be calculated for this period of gestation using the formula previously derived (1): safety factor = transfer of Na^{24} per gram fetus per hour (corrected) × 24 hrs. × 100 ÷ equilibrium con-

centration Na^{24} (corrected) × daily per cent weight increase. This calculation gives a safety factor of 100 at or near term. An analysis of the Na of maternal plasma and fetal ash of a fetus of about the same weight as the smallest shown in figure 4 gave a value for the ratio of concentration of Na in the fetus to that in the maternal plasma of 0.5. Using this value, the safety factor for the fetus of 70 grams is 28.

Discussion. The few observations (4) which have been made on the morphology of the placenta of the goat indicate that it is like that of the sheep and the conclusions drawn from the more numerous studies on the sheep have consequently been assumed to hold for the goat. The best evidence (4, 5, 6) leads to the view that within the cotyledonary placenta

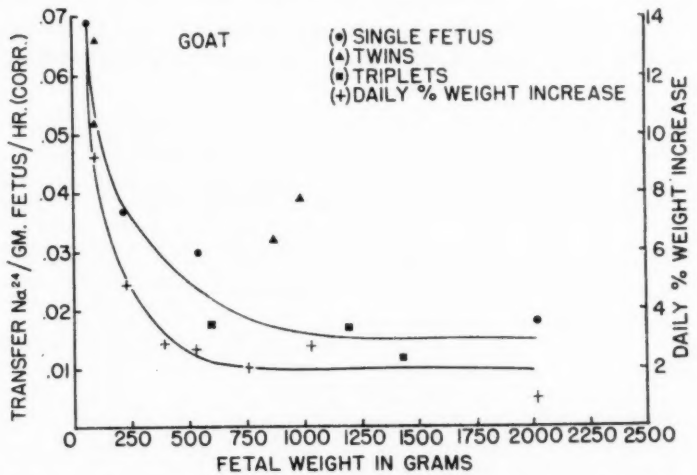


Fig. 4. Comparison of curve describing variation of daily per cent weight increase with variation of fetal weight and curve describing variation of transfer rate per unit weight of fetus with variation of fetal weight.

of these two animals, the maternal and fetal circulations are separated by endothelium of maternal blood vessels, maternal connective tissue, chorionic epithelium and mesenchyme, and endothelium of fetal blood vessels. In Grosser's classification (7), these are the characteristics of the group of placentae designated as syndesmochorial.

During the last half or third of pregnancy, several changes have been described within the cotyledonary areas. According to Assheton (5), the chorionic epithelium in the sheep consists of two layers, the outer of which is syncytial. The syncytial layer apparently does not completely disappear but becomes discontinuous in late pregnancy. Andresen (4) has also noted a thinning of the chorionic epithelium. In addition, as gestation proceeds, the fetal villi branch repeatedly and this leads to a reduction

of the connective tissue lying between their epithelium and the endothelium of maternal blood vessels. These morphological changes are reflected in the experimental data which show a three or fourfold increase in transfer rate from the ninth or tenth week to about the nineteenth and twentieth weeks of pregnancy.

As in the cases of the guinea pig, cat and rat, the curve describing the rate at which Na^{24} is supplied to a unit weight of the fetus of the goat is similar to the curve describing the relative growth rate of the goat fetus at various parts of gestation (fig. 4). The comparison of these two curves gives a measure of the adaptation of the placenta to fetal needs, for when a unit weight of fetus is reproducing itself relatively rapidly there is a correspondingly large demand for the transfer of substances across the placenta to each unit weight of fetus. It has been tentatively proposed (1, 2, 3) that a fundamental principle underlying change in placental transfer during the gestation period is that the rate of placental transfer to a unit weight of fetus shall vary as does the relative growth rate of the fetus. The degree to which this hypothesis is satisfied by the data on the goat has been shown above by a comparison of the ordinates of the relative growth curve and the curve of transfer rate of Na^{24} per gram of fetus.

TABLE 1

Comparison of placentae of cat and goat; and relationship between transfer rates of Na^{24} per gram fetus and relative growth rates of cat, goat and guinea pig

Values are those at the mid-point of the indicated period of gestation. Values for guinea pig and cat are taken from previously published data (1, 2).

	PERIOD OF GESTATION IN TENTHS OF TOTAL:			
	0.6-0.7	0.7-0.8	0.8-0.9	0.9-1.0
Ratio, transfer rates/gm. placenta, cat to goat	0.95	1.5	1.8	1.7
Ratio relative growth rates, cat to goat	5.1	4.6	3.8	3.0
Ratio transfer rates/gm. fetus, cat to goat	2.6	2.4	2.4	2.7
Ratio relative growth rates, guinea pig to goat	4.1	4.6	4.0	4.0
Ratio transfer rates/gm. fetus, guinea pig to goat	5.2	5.7	5.9	5.7

Not only is the relative growth rate of the fetus of a particular animal to be related to the rate of supply of a substance to a unit weight of fetus but the same sort of relationship between fetuses of different animals may be demonstrated. In table 1 the ratios of the relative growth rates of the fetuses of guinea pig and goat are given at different parts of gestation. Values are also given for the ratios of the transfer rates per gram of fetus. These two sets of ratios are remarkably alike. The values for the ratios of the relative growth rates of fetuses of cat and goat deviate more from the ratios of rates of transfer of Na^{24} per gram of fetus (table 1).

Table 1 also gives a comparison of the rates of transfer across unit weights of the placentae of the cat (2) and goat. The transfer rates per unit weight of placenta are alike in the sixth tenth of pregnancy and, indeed, the transfer rate of the cat's placenta at no time appears to be more than double that of the goat's. This is a difference not exceeding that found between placentae belonging to the same morphological group (3). From the viewpoint of rate of placental transfer as judged by Na^{24}, therefore, it appears likely that the syndesmochorial placenta of the goat may be placed in the same group as the endotheliochorial placenta of the cat. All other structures placed between the fetal and maternal circulations in the two placentae being considered more or less alike, it is not surprising that the additional maternal connective tissue of the cotyledons of the goat should have little measurable effect upon the transfer rate. Caution must be used in applying these results on the goat to the syndesmochorial placentae of other ruminants as considerable variation has been noted in the structure of the placentae of this group.

In the analysis of the observations, it will be noted that no account has been taken of the intercotyledonary placenta. This is epitheliochorial (5) and there is reason to believe that it affects in no important way the deductions drawn from the data.

These studies would have been impossible without the coöperation of Dr. Baldwin R. Curtis of the Department of Physics, Harvard University. We are grateful to him and we are indebted to the facilities offered by the Harvard cyclotron for the samples of radiosodium.

SUMMARY

1. The rates of placental transfer per unit weight of placenta have been measured with Na^{24} from a gestation age of about nine weeks until term. There is a three or fourfold increase in transfer rate from the ninth week to about the nineteenth and twentieth weeks of pregnancy.

2. The relative growth curve of the goat fetus is similar to the curve of rate of transfer of Na^{24} to a unit weight of fetus at different periods of pregnancy.

3. The placenta of the goat belongs to the syndesmochorial group. Its rate of transfer of Na^{24} per gram of placenta is of the same order of magnitude as that of the endotheliochorial placenta of the cat at comparable stages of pregnancy.

REFERENCES

(1) Flexner, L. B. and H. A. Pohl. This Journal **132:** 594, 1941.
(2) Pohl, H. A. and L. B. Flexner. J. Biol. Chem. **139:** 163, 1941.
(3) Flexner, L. B. and H. A. Pohl. J. Cell. and Compar. Physiol. **18:** 93, 1941.
(4) Andreson, A. Morphol. Jahrb. **57:** 410, 1927.
(5) Assheton, R. Phil. Trans. Roy. Soc. London, Series B **198:** 143, 1906.
(6) Grosser, O. Frühentwicklung, Eihautbildung und Placentation. 1927, Munich.

THE TRANSFER OF RADIOACTIVE SODIUM ACROSS THE PLACENTA OF THE RABBIT

LOUIS B. FLEXNER AND HERBERT A. POHL

From the Department of Embryology, Carnegie Institution of Washington, Baltimore, Md.

Accepted for publication July 7, 1941

In Grosser's classification (1) the placenta of the rabbit belongs to the hemochorial group. It is included in this series of investigations on placental transfer because the rabbit is widely used in studies on fetal physiology; and because, by comparison with the results obtained from the hemochorial placentae of the guinea pig (2) and rat (3), it will yield further evidence of the degree of variation in placental transfer among members of the same morphological group.

EXPERIMENTAL PROCEDURE. The general experimental procedure has previously been fully presented (2, 4). Radioactive sodium (Na^{24}), present as the chloride, was prepared by use of the Harvard cyclotron. Approximately 2 microcuries were taken for injection into an ear vein of a pregnant animal in all but the earliest stages of pregnancy when as much as 10 microcuries were used. The radioactivities of fetal ash and maternal plasma were measured with the pressure ionization chamber-string electrometer (2). Ether was given just before delivery of the fetuses by Caesarean section.

The units of radioactivity which have been used are those previously given (2, 3). The term "corrected" placed after values for Na^{24} means that these values have been corrected to a concentration of one beta-particle per second per cubic centimeter maternal plasma.

Measurements were made on 63 fetuses obtained from 17 pregnant animals. Twelve of these animals were of homogeneous breed and of known gestation age.

RESULTS. *Establishment of equilibrium between maternal plasma and fetus.* In the cases of the guinea pig (2), cat (4) and rat (3) the first experimental step was to establish the curve describing equilibration of the fetus with Na^{24} in the maternal plasma. This permitted the removal of the fetus at a time when its concentration of Na^{24}, derived from the maternal plasma, was increasing in a linear manner. In view of our previous experience, it was considered unnecessary to establish the equilibrium curve for the rabbit. Instead, the fetal concentration of Na^{24} at equilibrium was

obtained for several individuals and also measurement was made of the amount of Na^{24} which had been transferred to the fetus at the end of half an hour. At the end of half an hour, the fetus had attained a concentration of Na^{24} equal to one-fifth to one-sixth of the equilibrium value. This finding is like that in the rat. The routine procedure for measuring placental transfer in the rabbit was consequently the same as in the rat. Fetuses were removed at an exactly known interval, about half an hour, after intravenous injection of Na^{24} into the mother.

Rates of placental transfer. The change of placental weight with change of fetal weight is given in figure 1. The total Na^{24} transferred to the fetus per unit time as this varies with change of fetal weight is shown in figure 2. From these data have come the curves of figures 3 and 4.

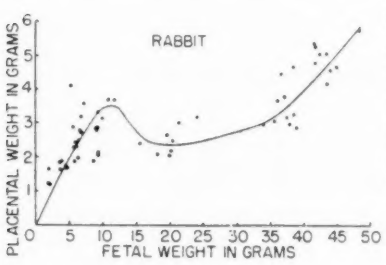


Fig. 1

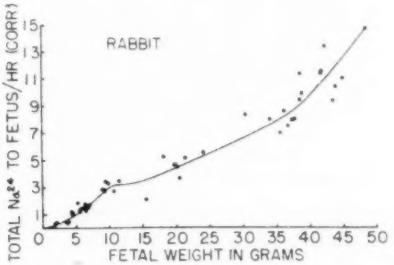


Fig. 2

Fig. 1. Variation of placental weight with variation of fetal weight. The gestation ages of the fetuses are given in figure 3.

Fig. 2. Variation of total hourly transfer of radiosodium to fetus with variation of fetal weight. The measured radioactivity of the fetal ash has in each instance been corrected to a concentration of one beta-particle per second per cubic centimeter of maternal plasma.

Figure 3 shows the changes in rate of transfer across a unit weight of placenta from the 18th day of gestation until term. At the 18th day the transfer rate is 0.25 unit and at the 30th day, 2.75 units. During these 12 days of pregnancy, consequently, the transfer rate per unit weight of placenta increases 11 times.

Figure 4 gives the rate at which Na^{24} is supplied from the maternal plasma across the placenta to each gram of fetus as this rate varies with fetal weight. This rate rises from a low value in the fetus of 4 grams to a peak which occurs at a fetal weight of about 8 grams (gestation age 22 days); then falls until term is approached when there is apparently a second rise. Figure 4 also gives the curve of the daily per cent weight increase. This has been calculated as previously explained (2) from our data on animals of known gestation age except for the first point corresponding to a fetal weight of 0.5 gram which has been taken from the data of Hammond

(5). The curve of relative weight increase as calculated from Hammond's data agrees closely with the curve derived from our data. The curve of

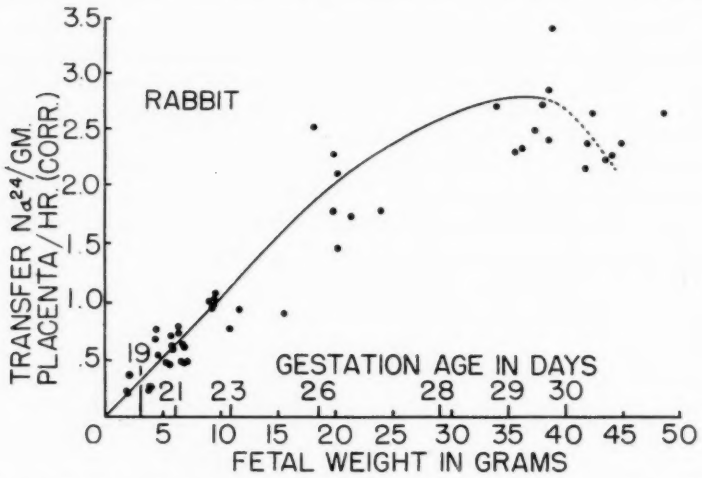


Fig. 3. Variation of transfer rate of Na^{24} per unit weight of placenta with variation of fetal weight or gestation age.

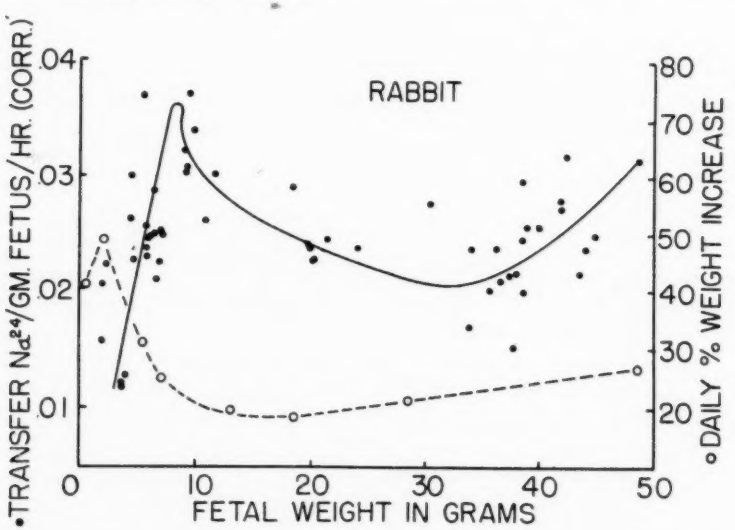


Fig. 4. Comparison of curve describing variation of daily per cent weight increase with variation of fetal weight and curve describing variation of transfer rate per unit weight of fetus with variation of fetal weight.

daily per cent weight increase has been found to be similar to the curve of rate of transfer of Na^{24} per gram of fetus in the case of other animals. In the case of the rabbit, however, the maximum in the curve of the daily per cent weight increase occurs at a fetal weight of 2 grams (gestation age 18 days) or about four days before the maximum is reached in the transfer rate of Na^{24} per gram of fetus. There is also a lag of approximately two days in the terminal rise in transfer rate per gram of fetus as compared to the terminal rise in the relative growth rate. Due to this difference in the time of occurrence of their maxima, the two curves are dissimilar up to a fetal weight of about 20 grams (gestation age 26 days). At greater fetal weights the correspondence between the two curves is more exact, the maximum variation in the ratios of their ordinates being from 2.7 to 4.

Fetal need for Na relative to supply across placenta. By using the transfer rate of Na^{24} per gram of fetus, the fetal equilibrium concentration of Na^{24} and the daily per cent growth rate of the fetus in an equation previously given (2, 3), the safety factor (ratio of the rate of supply of Na to

TABLE 1

Data necessary for calculation of safety factor

FETAL WEIGHT	TRANSFER Na^{24} PER GRAM FETUS PER HOUR (CORRECTED)	DAILY PER CENT WEIGHT INCREASE	EQUILIBRIUM CONCENTRATION; Na^{24} PER GRAM FETUS (CORRECTED)	SAFETY FACTOR
grams				
10	0.33	22	0.63	57
40	0.23	25	0.57	39

the fetus to the rate of accretion of Na by the fetus) for Na can be calculated. The values for the safety factor for the two fetal weights at which the equilibrium concentration of Na^{24} was determined are given in table 1.

Discussion. The transfer rate of Na^{24} per unit weight of placenta increases in the rabbit about 11 times from about the eighteenth to the thirtieth day of pregnancy. Results showing an increase in rate of placental transmission with increase in gestation age have also been reported by Lell, Liber and Snyder (6) who followed the urinary excretion of phenolsulphonephthalein by the mother after injection of the substance into the fetus; and by Rodolfo (7) who studied the transfer of antibodies across the placenta.

A considerable part of this increase in transfer rate is likely due to a true increase in placental permeability caused by changes in the tissues separating the maternal and fetal circulations. These changes as they occur on particular days of gestation have been studied by Mossman (8). At about the nineteenth day, the two circulations are separated by endothelium of fetal blood vessels and chorionic epithelium, i.e., the placenta is truly

hemochorial. Later stages of the placenta show progressive thinning of that chorionic epithelium and its progressive disappearance in many areas at which, consequently, the placenta is hemoendothelial. The evidence is clear that the barrier between the two circulations diminishes with advance of pregnancy and this change is to be correlated with the increase in transfer rate of Na^{24} per unit weight of placenta.

Circulatory changes which may affect the rate of placental transfer in the rabbit are not completely understood. Measurement of the rate of blood flow through the vessels of the pregnant uterus of the rabbit during the last half of pregnancy, however, gives evidence which suggests that during this period the rate of blood flow through the decidua basalis about doubles (9).

TABLE 2

Comparison of placentae of rabbit and guinea pig; and relationship between relative growth rates and transfer rates of Na^{24} per gram fetus of rabbit and guinea pig

Values are those at the mid-point of the indicated period of gestation. Values for guinea pig are taken from previously published data (2). The values on transfer rates in the rabbit have been corrected for the half-hour delivery time as previously explained (3).

	PERIOD OF GESTATION IN TENTHS OF TOTAL:		
	0.7–0.8	0.8–0.9	0.9–1.0
Transfer rate Na^{24}/gm. placenta, guinea pig	0.7	1.9	1.9
Transfer rate Na^{24}/gm. placenta, rabbit	0.6	1.8	2.1
Ratio relative growth rates, rabbit to guinea pig	2.5	2.4	3.3
Ratio transfer rates Na^{24}/gm. fetus rabbit to guinea pig	2.3	2.4	3.3

The rabbit placenta is the third member of the hemochorial group which has been studied with Na^{24}. A comparison of the transfer rate of Na^{24} per unit weight of its placenta with that of the guinea pig and rat gives a measure of the physiological variation among members of the same group. Such a comparison has been presented for the guinea pig and rat (3) and the transfer rates across unit weights of these two placentae have been found to be closely alike. The same sort of comparison is made between the rabbit and guinea pig placentae in table 2. It is apparent that the two placentae agree in their transfer rates per unit weight within narrow limits at comparable stages of pregnancy.

In all animals previously studied (2, 3, 4, 10) the curve of daily per cent weight increase has been similar to that curve of transfer rate of Na^{24} per gram of fetus. The rabbit is an exception in that the maximum in the curve of relative weight increase precedes by about four days the maximum

in the curve of transfer rate and the similarity between the two curves is not evident prior to a fetal age of 26 days. The significance of the relationship between these two curves as an index of the adaptation of the placenta to the needs of the fetus has previously been given (2, 3).

It has been pointed out that not only is there frequently a similarity in the relative growth curve and the curve of rate of transfer of Na^{24} per gram fetus during the development of a particular fetus but that the same sort of relationship may hold among fetuses of different animals (3, 10). A comparison of this latter kind is made between the fetuses of guinea pig and rabbit in table 2. At equivalent parts of the gestation period, the rabbit fetus reproduces its own weight between 2 and 3 times as rapidly as does the fetus of the guinea pig. In correspondence with its greater rate of growth, a unit weight of fetus of the rabbit receives across its placenta a supply of Na^{24} between 2 and 3 times that received by a unit weight of the fetus of the guinea pig.

It is a pleasure to express our gratitude to Dr. Baldwin R. Curtis who has generously supplied us with samples of radiosodium made with the Harvard cyclotron.

SUMMARY

1. The rates of placental transfer per unit weight of placenta have been measured in the rabbit from the eighteenth day of pregnancy until about term. There is an elevenfold increase in transfer rate per gram of placenta over this period.

2. The placenta of the rabbit belongs to the hemochorial group. The rate of transfer of Na^{24} across a unit of its weight agrees closely with that of the placentae of the guinea pig and rat which also are hemochorial.

3. The relative growth curve of the rabbit fetus is similar to the curve of rate of transfer of Na^{24} to a unit weight of fetus only after the twenty-fifth day of pregnancy. Prior to this, large changes in the relative growth curve anticipate similar changes in the curve of transfer rate per unit weight of fetus by about 4 days. Differences in the relative growth rates of guinea pig and rabbit are to be correlated with corresponding differences in the rate of transfer per unit weight of the respective fetuses.

REFERENCES

(1) Grosser, O. Frühentwicklung, Eihautbildung und Placentation. Munich, 1927.
(2) Flexner, L. B. and H. A. Pohl. This Journal **132:** 594, 1941.
(3) Flexner, L. B. and H. A. Pohl. J. Cell and Comp. Physiol. **18:** 93, 1941.
(4) Pohl, H. A. and L. B. Flexner. J. Biol. Chem. **139:** 163, 1941.
(5) Hammond, J. Trans. on the Dynamics of Growth **10:** 93, 1935.
(6) Lell, W. A., K. E. Liber and F. F. Snyder. This Journal **100:** 21, 1932.
(7) Rodolfo, A. J. Exper. Zool. **68:** 215, 1934.
(8) Mossman, H. W. Am. J. Anat. **37:** 433, 1926.
(9) Barcroft, J., W. Herkel and S. Hill. J. Physiol. **77:** 194, 1933.
(10) Pohl, H. A., L. B. Flexner and A. Gellhorn. This Journal **134:** 344, 1941.

THE EFFECT OF BARBITAL ANESTHESIA ON TEMPERATURE REGULATION[1]

ALLAN HEMINGWAY

From the Department of Physiology, The Medical School, University of Minnesota, Minneapolis

Accepted for publication July 10, 1941

In studying mammalian temperature regulation, the physiologist is handicapped by having to use anesthetized animals. The excitement and discomfort resulting from mild experimental procedures without anesthesia cause profound disturbances in temperature regulation. When anesthetics are used to eliminate these difficulties, the anesthetic will often introduce disturbing factors as detrimental as the excitement. Much of the past experimental work on temperature regulation has been done on animals under anesthesia and many problems now awaiting solution could best be performed in this condition. In order to evaluate and interpret such work, it is necessary to know to what extent anesthetics, which are commonly used for experimental physiology, depress temperature regulating mechanisms. Recently we have developed standardized tests for shivering and the vasomotor response in which measured stimuli have invoked a measured response in a controlled environment. These tests have been made on the same dog with and without anesthesia in order to obtain quantitative information on the depression caused by the barbital anesthetics on the response to cold.

It is well known that many anesthetics depress temperature regulatory functions. The drop of body temperature in the usual laboratory environment which follows anesthesia has been observed by many investigators but quantitative investigations on the extent, duration and functional type of depression are practically non-existent. Deuel, Chambers and Milhorat (1926) anesthetized dogs with amytal and noted a slight fall in oxygen consumption rate which, in the majority of the experiments, was less than 10 per cent of the basal value while the body temperature was reduced 2 to 3 degrees. Eddy (1929) found that diethyl barbituric acid, 42 mgm. per kilo by stomach tube, reduced the body temperature of cats two hours after anesthesia 0.1 to 0.5 degree. Ellis and Barlow (1924–5) used higher doses of barbital, 182 mgm. per kilo intraperitoneally, and found a de-

[1] Technical assistance in this investigation was furnished by Onni Overhouse and George Cordes of the Works Progress Administration, Official Project number 665-71-3-69, subproject number 205.

pression of body temperature which, during the course of anesthesia, dropped to values as low as 3 degrees below normal and returned to normal after 48 hours. Page and Coryllos (1926) found that amytal anesthesia caused a slight drop in blood pressure of dogs, and Lepper (1926) observed that small doses of veronal caused a constriction of ear vessels while larger doses produced dilatation. These few observations, taken from papers in which temperature regulation was not the primary interest of investigation, are in agreement with our observations that barbital anesthesia causes a drop in body temperature with peripheral vasodilatation and impaired shivering in the usual laboratory environment. These previous investigations, insofar as they are concerned with temperature regulation, are far from being complete. In many cases room temperature was not controlled nor were skin and rectal temperatures followed through the course of anesthesia and correlated with temperature regulatory mechanisms.

In our investigations sodium pentobarbital and amytal have been used in dosages suitable for surgical anesthesia and physiological experimentation. These anesthetics have been chosen on account of their ease and convenience of administration and their extensive use by physiologists. Control experiments have been made in which normal responses have been measured without anesthesia. Identically similar experiments were performed on the same dogs under anesthesia. The reactions which have been measured are shivering and the vasomotor response.

EXPERIMENTAL. *Part I. Depression of shivering and vasomotor control with anesthetic doses of barbitals.* Four short haired bull terrier type dogs weighing 10 to 15 kilos were carefully chosen and trained for testing without anesthesia. The animals were placed on a mechanical shivering recorder, as described by Hemingway (1940), in an air conditioned room in which the air temperature was 22.0 $\pm$ 0.5 degrees, the relative humidity 50 $\pm$ 5 per cent, and the air velocity 25–40 feet per minute. Diathermy electrodes were placed on the dog, one electrode being beneath the recumbent animal while the other was placed on the upper flank. Temperatures of the skin of the ears, foreleg, thorax, beneath the diathermy electrodes, and of the rectum were measured by thermocouples. The dog was trained to lie quietly on the recorder and as a result of resting in this cool environment, the skin and rectal temperatures fell, producing first vasoconstriction, noted by a rapid fall of ear temperature, and then shivering which prevented a further fall in temperature. When the steady state was reached the diathermy current was turned on and the dog heated at a rate equal to the b.m.r. As the heating progressed shivering stopped and finally vasodilatation occurred. Temperatures and shivering intensity were plotted against time, as shown in figure 1. Each experiment lasted 3 to 8 hours.

The same experiment was performed on the same dog on another day after being anesthetized. Sodium pentobarbital was given both intravenously-intramuscularly and intraperitoneally in doses of 35 mgm. per

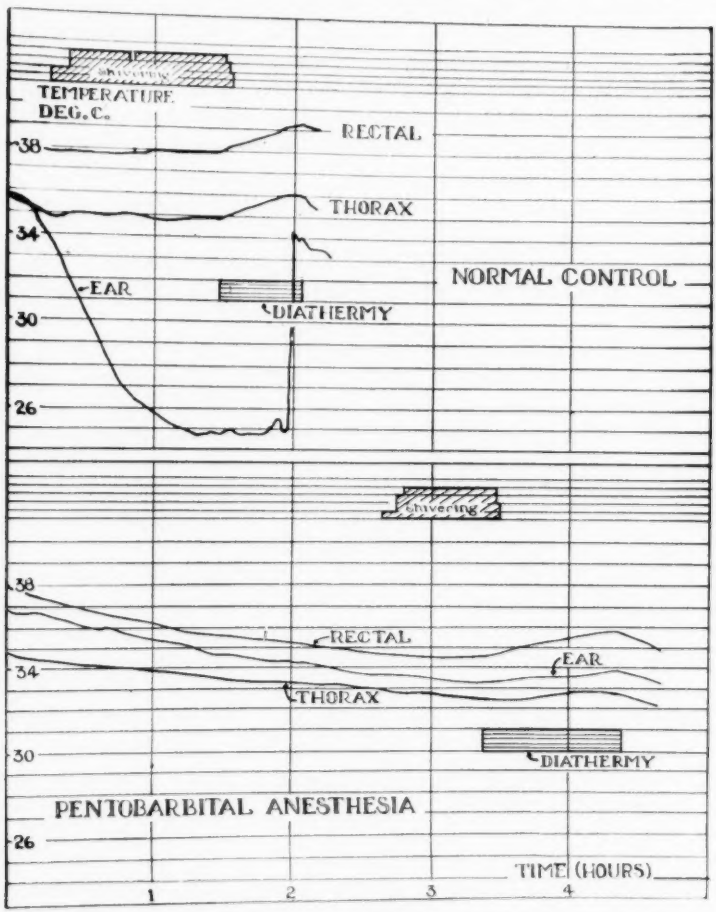


Fig. 1. The change of skin and rectal temperatures of a normal and an anesthetized dog in a cool room before and after diathermy heating. Shivering is indicated on an arbitrary scale with no shivering indicated as 0 and most intense shivering as 5.

kilo. When given intravenously two-thirds of the full dose was injected at once with the remainder being injected intramuscularly 45 minutes later. When administered intraperitoneally the full dose was injected at one time. One or two experiments were made on each mode of anesthe-

tization. The same procedure was used when sodium amytal was given except that the total dosage was 55 mgm. per kilo. On each dog at least 4 control experiments were performed.

Part II. Duration of temperature regulatory depression with anesthetic doses of barbitals. In order to investigate the extent of recovery of shivering and vasomotor control during the 3 to 4 hour period of anesthesia, a series of experiments was made in which the following procedure was adopted for one dog (B). The dog was placed on the shivering recorder in the constant temperature-constant humidity room, and the thermocouples and diathermy electrodes were attached. The diathermy current was started and regulated to maintain the body temperature constant with shivering and vasoconstriction being prevented. After 30 minutes of rest sodium pentobarbital, 35 mgm. per kilo, was injected intraperitoneally in a single dose. The diathermy current was continued and varied to keep the body temperature from falling under anesthesia. After a time interval of from 0 to 240 minutes the diathermy current was discontinued and cooling was allowed to proceed while measurements of temperature and shivering were made. In a series of experiments the heating period between injection of the anesthetic and cessation of diathermy was set at 0, 30, 60, 90, 120, 180 and 240 minutes. Thus the animal was not allowed to cool until after periods of anesthesia having these duration times. For each experiment a time-temperature graph similar to those of figure 1 was made.

RESULTS. In figure 1 are two graphs, one showing the response of a normal dog to cold followed by diathermy heating, and the other graph the response of the same dog under pentobarbital anesthesia in an identical experiment. A normal dog responds to a cool environment by peripheral vasoconstriction, which is most marked in the ear vessels. The result is a drop in ear temperature to 25 or 27 degrees. Simultaneously, or a few minutes later, shivering occurs, after which the body temperature remains at a constant level. In a dog anesthetized with either of the two barbiturates, both the vasomotor and shivering mechanisms are partially or completely paralyzed. In the experiment recorded by the lower graph of figure 1, which indicates marked temperature regulatory impairment, peripheral vasoconstriction did not occur in spite of a drop in rectal temperture of several degrees. Shivering did occur but the temperature thresholds were depressed several degrees.

There was a considerable variation in response between different anesthetized animals and the same animal on different days. The usual temperature-time curves during anesthesia were intermediate between the two extremes represented in figure 1, i.e., the normal response and that of the deeply narcotized animal with severe temperature regulatory paralysis.

Part I. Shivering and vasomotor depression. For each dog at least 4 con-

trols and 4 anesthetic experiments were performed. A graph similar to those of figure 1 was drawn and the significant data taken from each graph is listed in tables 1 and 2. For the 4 control experiments, maximum,

TABLE 1

Shivering

DOG	ANES. METHOD	RECTAL TEMPERATURE				SKIN TEMPERATURE (THORAX)		
		Shivering		Min. temp. reached	Time to reach min. temp.	Shivering		Min. temp. reached
		Starts	Stops			Starts	Stops	
A (max.)	Con.	36.8	37.2	36.8	70	34.2	34.0	33.5
A (min.)	Con.	35.9	36.2	35.9	40	32.5	33.2	32.5
A (ave.)	Con.	36.4	36.8	36.4	55	33.5	33.7	33.1
A	NP	33.0	38.3	33.0	150	30.1	33.8	30.1
A	NV	34.0	38.7	34.0	135	30.8	33.6	30.7
A	AP	N.S.	N.S.	32.4	78	N.S.	N.S.	29.6
A	AV	33.6	38.3	33.1	85	30.1	29.7	29.4
B (max.)	Con.	38.1	38.5	37.9	100	34.8	35.5	34.6
B (min.)	Con.	37.6	38.0	37.2	10	34.3	34.3	33.5
B (ave.)	Con.	37.8	38.2	37.6	60	34.6	34.8	34.2
B	NP	34.4	36.6	34.4	105	32.3	34.4	32.3
B	NV	35.0	36.1	35.0	165	31.2	31.5	31.2
B	AP	36.4	39.8	36.1	80	33.1	36.6	33.1
B	AV	N.S.	N.S.	32.5	70	N.S.	N.S.	30.1
C (max.)	Con.	38.3	38.6	38.2	105	34.2	35.0	34.0
C (min.)	Con.	38.0	38.2	37.8	50	33.4	33.4	33.3
C (ave.)	Con.	38.1	38.4	38.0	75	33.8	34.3	33.7
C	NP	37.4	40.7	37.3	70	33.9	35.2	33.1
C	NV	36.1	36.3	36.1	50	31.1	31.1	31.1
C	AP	36.6	38.1	36.1	58	33.0	34.1	32.6
C	AV	36.1	36.7	36.1	65	32.0	31.5	31.1
D (max.)	Con.	37.9	38.3	37.9	95	35.4	35.3	35.7
D (min.)	Con.	37.7	38.1	37.4	40	33.6	34.9	33.9
D (ave.)	Con.	37.8	38.2	37.7	63	34.8	35.1	34.5
D	NP	36.0	37.9	36.0	115	32.0	32.8	31.8
D	AP	35.5	36.0	34.9	135	32.1	32.5	31.6
D	AV	35.5	37.1	35.2	80	31.0	31.7	31.0

N.S., no shivering; Con., control, no anesthesia; N, pentobarbital Na; A, amytal Na; P, intraperitoneal injection; V, intravenous-intramuscular injection.

minimum and mean values are given. The letters N and A denote sodium pentobarbital and amytal, respectively, while P and V denote intraperitoneal and intravenous-intramuscular administrations, respectively. Table 1 contains shivering data and table 2 vasomotor data. Table

1 contains the rectal and thoracic skin temperatures at which shivering started during cooling and stopped after application of diathermy. The minimum temperatures reached and the times required to reach minimum temperatures are also given. Shivering started usually within 1 to 2 hours after anesthesia with rectal temperatures of 33 to 36 degrees while

TABLE 2

Vasomotor

DOG	ANES. METHOD	VASOCONSTRICTION			VASODILATATION		
		Rectal t.	Thorax t.	Resting time	Rectal t.	Thorax t.	Heating time
A	Con. (max.)	37.1	34.5	58	38.0	34.3	92
A	Con. (min.)	36.1	32.9	31	37.2	33.2	31
A	Con. (ave.)	36.6	33.5	44	37.7	33.6	44
A	NP	33.5	30.8	42	38.8	33.8	112
A	NV	No vasoconstriction					
A	AP	32.6	30.0	68	36.5	31.4	100
A	AV	36.1	32.4	40	37.7	30.6	151
B	Con. (max.)	38.6	35.7	28	39.0	37.6	103
B	Con. (min.)	38.0	34.6	15	38.7	34.7	23
B	Con. (ave.)	38.2	35.2	22	38.9	35.5	77
B	NP	35.7	32.6	60	37.0	33.1	54
B	NV	34.9	31.3	140	38.3	33.1	240
B	AP	36.5	33.5	58	39.8	36.4	100
B	AV	37.3	33.6	27	38.5	33.9	115
C	Con. (max.)	38.5	34.6	62	39.0	35.1	38
C	Con. (min.)	38.1	33.6	27	38.7	34.8	13
C	Con. (ave.)	38.4	34.3	42	38.9	34.7	21
C	NP	37.4	35.0	45	39.7	35.1	48
C	NV	36.3	32.3	65	38.6	32.4	240
C	AP	36.3	32.9	52	38.1	34.1	25
C	AV	36.7	32.1	28	38.1	31.9	110
D	Con. (max.)	38.3	35.5	85	39.0	36.3	63
D	Con. (min.)	37.5	33.5	43	38.5	33.6	3
D	Con. (ave.)	37.9	35.1	62	38.7	35.2	25
D	NP	No vasoconstriction					
D	AP	35.2	31.9	95	36.3	32.6	32
D	AV	35.4	31.4	73	38.0	32.3	19

the normal rectal threshold temperature for shivering is 35.9 to 38.3 degrees. Dog D was unfortunately killed as a result of a dog fight before the last experiment was performed; hence the results of intravenous pentobarbital anesthesia for dog D are missing.

Table 2 contains the vasomotor data. The rectal and skin thoracic

temperature thresholds are given at which peripheral vasoconstriction occurred as a result of resting in a cool room and at which vasodilatation occurred when the animals were subsequently heated by diathermy at a heating rate equal to the b.m.r. In the column designated as "heating time" the time in minutes of diathermy is given which was required to produce vasodilatation. Since the heating rate was kept constant, this time interval is proportional to the heat dosage required to produce vasodilatation.

Part II. Duration of temperature regulatory depression. The significant data, collected from the time-temperature graphs of experiments in which the effect of duration of anesthesia on cold response depression was investigated, are given in table 3. The "anesthetic diathermy time" is the time interval between injection of the anesthetic and cessation of the diathermy current. It is the time interval of anesthesia before cooling in which the rectal temperature was prevented from falling by diathermy. The table contains both rectal and skin thoracic temperatures at which vasoconstriction and shivering occurred as well as the time interval, "cooling time", between cessation of diathermy and the onset of shivering or vasoconstriction. In the normal unanesthetized dog used for these tests, vasoconstriction occurred with a rectal temperature of 38.2 degrees and a thoracic skin temperature of 34.0 degrees after 13 minutes of cooling. Shivering occurred after 27 minutes of cooling when the rectal temperature had reached 37.9 degrees and the skin thoracic temperature 34.0 degrees.

TABLE 3

Duration of temperature regulatory depression by sodium pentobarbital

CONDITION	VASOCONSTRICTION			SHIVERING		
	Rectal temp.	Thoracic temp.	Cooling time	Rectal temp.	Thoracic temp.	Cooling time
Control—no anesthetic	38.2	34.0	13	37.9	34.0	27
Anes diathermy time = 0	36.1	31.5	40	31.4	28.1	343
	34.7	30.8	105	32.5	29.2	230
Anes diathermy time = 30	35.6	31.6	63	32.4	29.1	280
Anes diathermy time = 60	35.4	31.5	100	33.6	30.1	215
Anes diathermy time = 90	36.3	32.7	48	34.8	31.6	132
Anes diathermy time = 120	35.4	31.6	90	34.1	30.8	158
Anes diathermy time = 150	36.0	32.6	78	35.6	32.4	118
Anes diathermy time = 180	†	†	†	34.7	32.0	164
Anes diathermy time = 240	*	*	*	*	*	*

* Dog awakened when heating discontinued. Running movements and struggling prevented a fall in temperature and obscured shivering.

† Ear vessels constricted while dog was being heated.

As a result of anesthesia the cooling time is prolonged and the threshold temperatures lowered. There is only slight recovery of shivering and vasoconstriction 2 to 3 hours after anesthesia. For example, immediately after anesthesia shivering commenced at a rectal temperature of 31.4 to 32.5 degrees while 2 to 3 hours after anesthesia shivering commenced with rectal temperatures of 34.1 to 35.6. The animal awakens before there is recovery from the depression of shivering and thermal vasoconstriction.

Discussion and conclusion. For experimentation with dogs barbital anesthesia is widely preferred by physiologists. The anesthetic can be easily administered and anesthesia after a single dose lasts 2 to 4 hours. There is no appreciable effect of the barbital anesthetics on blood pressure and respiration is only slightly depressed. There is no appreciable fall of basal metabolic rate provided the body temperature is maintained. The main depressant autonomic effect is on temperature regulation. Our experiments were performed to determine how extensive this depressant action is and to what extent a physiologist is justified in using the barbitals for acute experiments on temperature regulation.

The results indicate that there is a considerable depression of both peripheral and rectal threshold temperatures at which shivering and thermal vasoconstriction commence. The depression amounts to as much as 1 to 5 degrees. Since, according to current theories, the mechanisms which protect against cold are activated when peripheral and central temperatures fall below a definite threshold value, the lowering of this threshold indicates considerable impairment by the anesthetics of the physiological temperature regulating apparatus. However, in only about ten per cent of the experiments was peripheral vasoconstriction or shivering completely inhibited. This indicates a lack of precision of control but not complete absence of temperature regulation.

There was considerable variability with the same experiment repeated on different days using the same dog. Due to this variability there was no detectable difference in the depression of temperature regulation caused by sodium amytal or pentobarbital, nor was there any difference between the intravenous and intraperitoneal methods of administering the anesthetic.

The depression of temperature regulation against cold caused by these barbiturates lasted 3 to 4 hours and exceeded the period of anesthesia. There was only a slight recovery near the end of the anesthetic period.

SUMMARY

In a cool environment of 22.0°C. and 50 per cent relative humidity, trained dogs were allowed to rest until shivering and peripheral vasoconstriction had occurred. The animals were then heated by diathermy at a rate equal to the b.m.r. until shivering was inhibited and vasodilatation

had occurred. The experiments were performed with and without amytal and pentobarbital anesthesia. Shivering was measured on a mechanical recorder and skin and rectal temperatures were measured by thermocouples. It was found that shivering and vasomotor temperature thresholds were suppressed by the anesthetics 1 to 5 degrees, and the small differential temperature range of normal animals was replaced by a differential having a 3 to 5 fold range as a result of anesthesia. Barbital anesthesia in doses required for surgical procedures suppresses the responses to cold but does not completely inhibit them. The order of suppression for temperature regulatory processes in barbital anesthesia is, in order of decreasing severity, 1, body temperature; 2, shivering; 3, vasomotor reflexes.

REFERENCES

DEUEL, H. J., W. H. CHAMBERS AND A. T. MILHORAT. J. Biol. Chem. **69:** 249, 1926.
EDDY, N. B. J. Pharmacol. and Exper. Therap. **37:** 273, 1929.
ELLIS, M. M. AND O. W. BARLOW. J. Pharmacol. and Exper. Therap. **24:** 259, 1924–25.
PAGE, I. H. AND P. CORYLLOS. J. Pharmacol. and Exper. Therap. **37:** 273, 1929.
LEPPER, L. Pflüger's Arch. **204:** 498, 1924.
HEMINGWAY, A. This Journal **128:** 736, 1940.

AN ANALYSIS OF HYPOTHALAMIC CARDIOVASCULAR CONTROL

R. F. PITTS,[1] M. G. LARRABEE AND D. W. BRONK

From the E. R. Johnson Foundation, University of Pennsylvania, Philadelphia, and the Department of Physiology, New York University College of Medicine, New York

Accepted for publication June 19, 1941

The buffer reflexes, centrally integrated in the lower brainstem, serve to adjust the rate of the heart and the caliber of the vessels so that a sufficient volume flow of blood is maintained at a satisfactory pressure. But lability of control of the cardiovascular system is necessary to enable the organism to meet environmental stresses. A rise in blood pressure, an increase in heart rate, and a redistribution of blood, provide for the increased activity so commonly a part of affective behavior. A shift of blood between viscera and skin provides a means of dissipating or conserving heat. The dominant rôle assigned to the hypothalamus in emotional expression (Bard, 1929), and in thermal regulation (Ranson and Magoun, 1939) suggests that this central nervous level contributes a very necessary element of lability to medullary cardiovascular control.

Karplus and Kreidl (1928), Kabat, Magoun and Ranson (1935), Ectors, Brookens and Gerard (1938) and others have shown that electrical stimulation of the hypothalamus in the anesthetized animal leads to an increase in heart rate and a rise in blood pressure. Morrison and Rioch (1937) and Magoun (1938) demonstrated that these cardiovascular responses are independent of descending pathways from the cerebral cortex and result from activation of projection systems which take origin within the hypothalamus. The course of the descending hypothalamic connections, through the tegmentum and central gray of the mesencephalon, pons and into the medulla, brings them in close relation with those medullary centers which reflexly control the heart and blood vessels (Magoun, Ranson and Hetherington, 1938; Wang and Ranson, 1939). Through these connections it is possible that the hypothalamus might modify the basic reflex pattern laid down in the medullary centers for cardiovascular control.

One of the most effective methods of analysis of this problem is a study of the discharge of impulses initiated in sympathetic motor neurones by stimulation of the hypothalamus, and a consideration of how this activity is integrated into the reflex control of the cardiovascular system. Such a

[1] Fellow of the Rockefeller Foundation.

procedure has distinct advantages as compared with observation of the response of effector organs. Because of the latency and inertia of these organs, they cannot be used to investigate the precise nature of rapidly occurring neural processes. We have therefore recorded the activity in peripheral sympathetic nerve trunks supplying heart and blood vessels during stimulation of the hypothalamus and have correlated that activity with the response induced in the cardiovascular system. Even the discharge of impulses in a peripheral nerve trunk is not entirely suitable and we have accordingly carried out certain portions of our investigation on single units.

METHODS. Experiments were performed on cats anesthetized with 20 to 30 mgm. per kgm. of nembutal, or 50 mgm. per kgm. of chloralosane, given intravenously. Bipolar needle electrodes were introduced into the hypothalamus by means of the Horsley-Clarke stereotaxic instrument, and brief repetitive condenser discharges delivered at controlled intensities and frequencies. At the end of each experiment small electrolytic lesions were placed to mark the position of the electrode tips in the brain. The animal was perfused with formalin, the brain removed, and after a period of hardening, the brain was sectioned grossly to determine the site of stimulation. In representative experiments, the brains were embedded in nitrocellulose, sectioned at 50 microns, stained by the Weil method and examined to determine the exact placement of the electrodes.

Two nerves were used for recording sympathetic neural activity, the cervical sympathetic and the inferior cardiac nerve. The inferior cardiac nerve was approached by removal of the chest wall and careful dissection of the mediastinal contents, the animal being maintained with artificial respiration. The cervical sympathetic nerve was freed in the neck and sectioned peripherally.

Because it is difficult to determine variations in nervous activity in records of the summated action potentials from the many fibers of a nerve trunk, we have in general employed small twigs of the cardiac nerve split off from the main body. Single fibers of the cervical sympathetic nerve were obtained by careful dissection in a moist chamber until visual and aural examination of the amplified action potentials indicated that only a single functional unit remained active. In all experiments the animal was kept in a shielded room maintained near 37°C. and 100 per cent humidity.

Action potentials were suitably amplified with a capacity coupled amplifier and recorded with a General Electric oscillograph and a bromide paper camera. A few records were made with a cathode ray oscillograph. Simultaneous records of blood pressure were made in a number of experiments with an optical manometer connected with either the femoral or carotid arteries.

RESULTS. *Nature of activity in the cardiac nerve.* The spontaneous activity which may be recorded from the inferior cardiac nerve is typically grouped into smooth potential waves of relatively large amplitude (Bronk, Ferguson, Margaria and Solandt, 1936). These waves are frequently synchronous with the pulse and occur at a frequency of 2 or 3 a second. Such a pulse modulation of the discharge is dependent upon the integrity of the buffer nerves. Bronk (1934) has shown that it represents the tonic accelerator activity of the medullary cardiovascular centers, periodically inhibited by the discharge of pressure receptors located in the carotid sinus and aortic arch. The nature of this grouped activity in the inferior cardiac nerve is unaffected by removal of brainstem structures above the pons, and is in no wise dependent upon the hypothalamus (Bronk, Pitts and Larrabee, 1940).

Effect of hypothalamic stimulation on cardiac nerve discharge. Stimulation of the hypothalamus with low intensity, high frequency (150 per sec.)

Fig. 1. Increased discharge of impulses in the cardiac sympathetic nerve during hypothalamic stimulation at a frequency of 150 per second, followed by inhibition of spontaneous discharge. Arrows mark beginning and end of the period of stimulation. Upper tracing, arterial blood pressure. Time, $\frac{1}{5}$ second.

condenser discharges leads, after a brief latency of less than 0.1 second, to a marked increase in activity in the cardiac nerve. The abrupt onset of this activity as stimulation is begun (first arrow) and its equally abrupt cessation as stimulation is stopped (second arrow) is apparent from figure 1. In this experiment blood pressure began to rise after an interval of 1.5 seconds, continued to rise for 1 second after stimulation was stopped, and remained elevated for another 6 seconds or more. In contrast to the prolonged elevation of blood pressure, activity in the inferior cardiac nerve ceased abruptly as the stimulus was stopped, and for the succeeding 4 seconds all spontaneous discharge was abolished.

It is apparent that the postulated prolonged after-discharge from the hypothalamus following stimulation (Grinker and Serota, 1938) finds no support in our experiments on the anesthetized animal. Prolonged elevations of blood pressure which do occur in these experiments are obviously due to inertia of the effector system and not to sympathetic after-discharge.

Often, if the stimulus is prolonged or made more intense, a secondary

rise in blood pressure occurs some 10 or more seconds after the initial rise. This secondary rise, which may occur some seconds after the stimulus is stopped and at a time when all sympathetic activity is inhibited, is interpreted as being due to the release of adrenalin, described by Houssay and Molinelli (1925) and Magoun, Ranson and Hetherington (1937). In accord with the experience of Kabat, *et al.* (1935), the heart is but little

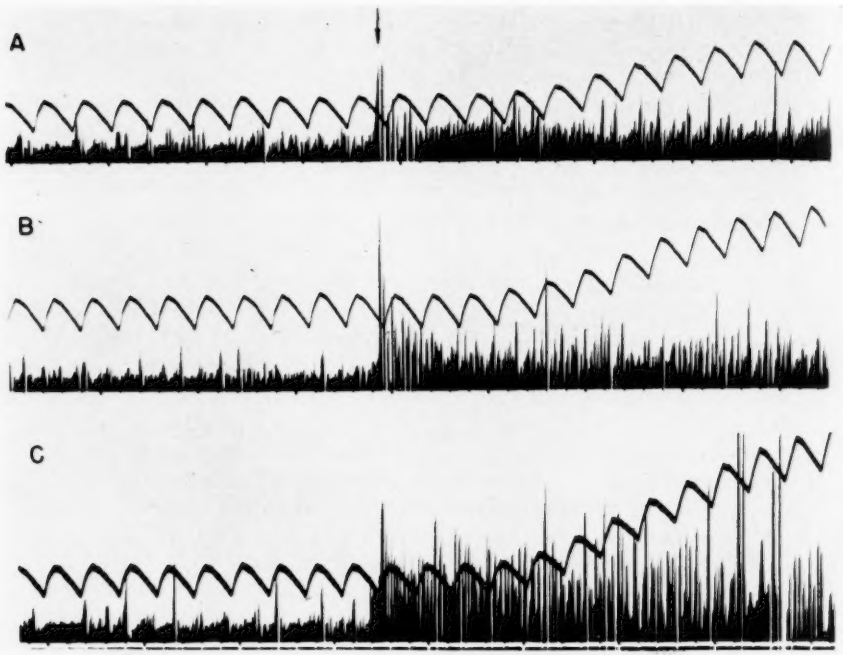


Fig. 2. Increased discharge of impulses in the cardiac sympathetic nerve and rise of blood pressure during hypothalamic stimulation at a frequency of 150 per second and relative intensities of 1 in A, 2 in B and 3 in C. Arrow marks the beginning of stimulation. Time, $\frac{1}{5}$ second.

accelerated, rarely more than 10 per cent, a fact no doubt correlated with the high heart rate and lack of vagal tone in the cat.

Factors controlling hypothalamic excitation and cardiovascular response. A. Stimulus intensity. An increase in intensity of hypothalamic stimulation accentuates those processes which have just been described. Figure 2 illustrates the results of stimulation of the hypothalamus at a constant frequency of 150 per second, but with increasing relative intensities of 1 in record A; 2 in record B; to 3 in record C. With increase in stimulus

strength, the discharge in the cardiac nerve progressively increased from A to C. The magnitude of the blood pressure rise and the steepness of its gradient increased in parallel manner with increasing stimulus strength.

Had the records of figure 2 been reproduced over a longer period, they would show from A to C a progressive increase in the time that spontaneous activity in the cardiac nerve was inhibited following cessation of stimulation. They would show an increased duration of the elevation of blood pressure, as well as an increased secondary rise in blood pressure, as a result of increased stimulus strength.

Records such as these illustrate well the effects of hypothalamic stimulation, but little concerning the mechanism by which they are achieved.

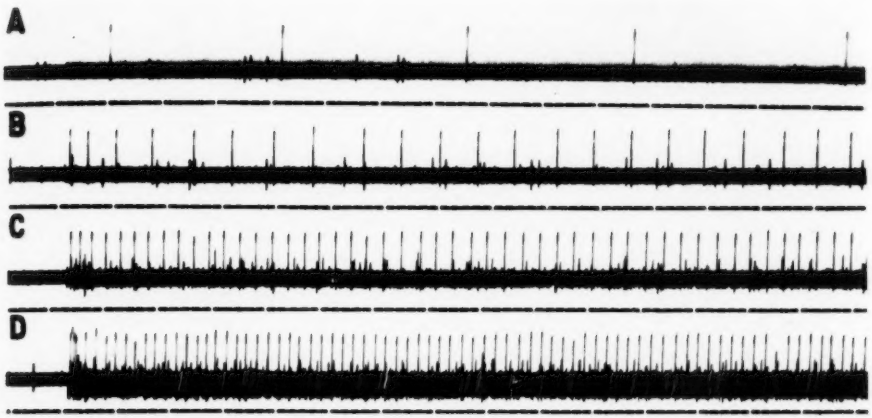


Fig. 3. Discharge of impulses in a single fiber of the cervical sympathetic nerve in response to hypothalamic stimulation at a frequency of 100 per second. Intensity progressively increased from A to D. Time, ½ second.

It is readily apparent that the number of messages carried by a nerve to the heart or blood vessels is greatly increased by hypothalamic stimulation; but so many messages are being carried by so many different fibers, that the fundamental content of any one message is obscured by the activity of the mass.

Accordingly, we have studied the nerve impulses carried by single nerve fibers dissected from the cervical sympathetic trunk. Records from such a preparation are presented in figure 3. Instead of the random mass discharge observed in figures 1 and 2 from a many fiber preparation, the single unit here illustrated responded rhythmically and repetitively to hypothalamic stimulation. The story which such a unit tells is a simple one; its one variable is frequency of response. As a result of increasing

strength of hypothalamic stimulation, such a single unit responds with increasing frequency of discharge as illustrated in records A to D. In this experiment the frequency of hypothalamic stimulation was maintained constant at 100 per second and the intensity altered. With an increase in stimulus intensity of some 2.5 times, the response frequency of the neurone varied from about 1 in 2 seconds to 9 per second.

We have arbitrarily selected for all our studies, single fibers which were not spontaneously active. Such units, after a period of a second or so of adaptation, maintain a very constant frequency of discharge in response to maintained hypothalamic stimulation. If a period of a half minute or more intervenes between two periods of stimulation, the response frequencies

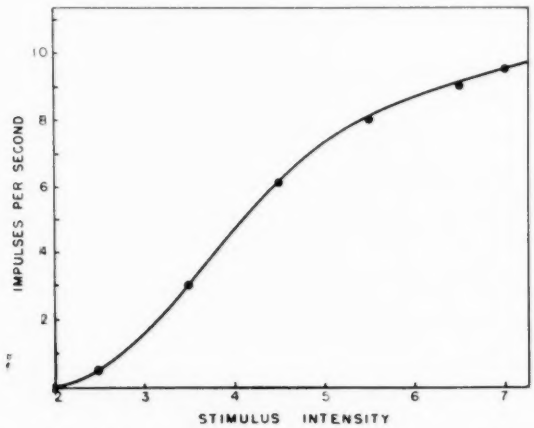


Fig. 4. The relation between frequency of discharge of impulses in a single fiber of the cervical sympathetic nerve and intensity of hypothalamic stimulation. Frequency of stimulation constant at 100 per second.

are reproducible. These facts make quantitation of our results in terms of impulse frequency an adequate method of presentation.

A plot of impulse frequency against stimulus intensity, taken from the entire series of records of which figure 3 is a part, is shown in figure 4. The relationship is smoothly curved and sigmoid in shape. The intensity units are arbitrary ones, but the highest intensity used is below that at which any significant motor response occurs, a limitation enforced by our methods of recording.

From these observations of the behavior of single fibers as the intensity of hypothalamic stimulation is increased, the records of figures 1 and 2 acquire added significance. The gross increase of activity observed in the cardiac nerve must, in part, be due to an increase in the number of impulses

carried by the constituent units making up that nerve. The temporal summation of those impulses at the effector organ produces a response which is graded according to impulse frequency, and hence according to intensity of hypothalamic stimulation. However, increase in intensity of hypothalamic stimulation not merely increases the frequency of discharge in a given unit, but also increases the number of active units as well, for their thresholds of excitation vary over a wide range. Examples of such increase in number of active units will be presented in a later section. Suffice it to say here, that magnitude of response in sympathetic activity is determined by variations in both the frequency of impulses in any one neural unit and in numbers of neural units active. Such a relation has been described by Adrian and Bronk (1928) for the gradation of somatic motor reflexes and by Bronk and Ferguson (1935) for the gradation of intercostal respiration.

B. *Placement of electrodes.* The intensity of hypothalamic excitation may be varied not only by alteration of stimulus strength, but also by changing the placement of the stimulating electrodes within the hypothalamus. The strengths used for hypothalamic stimulation have been low, as mentioned above, and spread of stimulus has been kept to a minimum by using bipolar electrodes, the tips of which were separated about 0.2 mm. The distance of current spread from the electrode tips is small under such conditions (Pitts, 1941).

In figure 5 is shown the response of two neurones, each identifiable by its characteristic spike potential. During each record, the hypothalamus was activated by the same low intensity stimulation at a frequency of 180 per second. From A to E, the electrode was progressively lowered through the hypothalamus in steps of 1 mm. Figure 6 A shows the position of the electrode at each level within the hypothalamus. To the right of the electrode track is given the frequency of discharge of the neurone whose spike potential is the larger; to the left is the letter identifying the level with a particular record of figure 5. In figure 6 B are presented the results of another experiment, in which a single fiber was activated from a number of levels on both the ipsilateral and contralateral sides of the hypothalamus. The relatively constant frequency of discharge to stimulation over the lower 3 mm. of the hypothalamus on both sides, coupled with the sudden decline in frequency as the electrode is raised above this level can only mean that the hypothalamic field, which is in functional relationship with a given preganglionic sympathetic neurone, is not only bilaterally distributed, but also has a considerable dorsoventral extent. This view is supported by a number of experiments in which the frequency of discharge of a given single preganglionic neurone has been determined for stimuli of various intensities applied to the two sides of the hypothalamus. A weak stimulus, which is just threshold for the side which is ipsilateral to the cervical

sympathetic neurone whose activity is being recorded, will usually excite that neurone if applied to the contralateral hypothalamus. If not, a slight increase in intensity will bring it to a threshold level.

The results presented so far may be most readily interpreted as indicating that a number of parallel pathways connect the hypothalamus with the

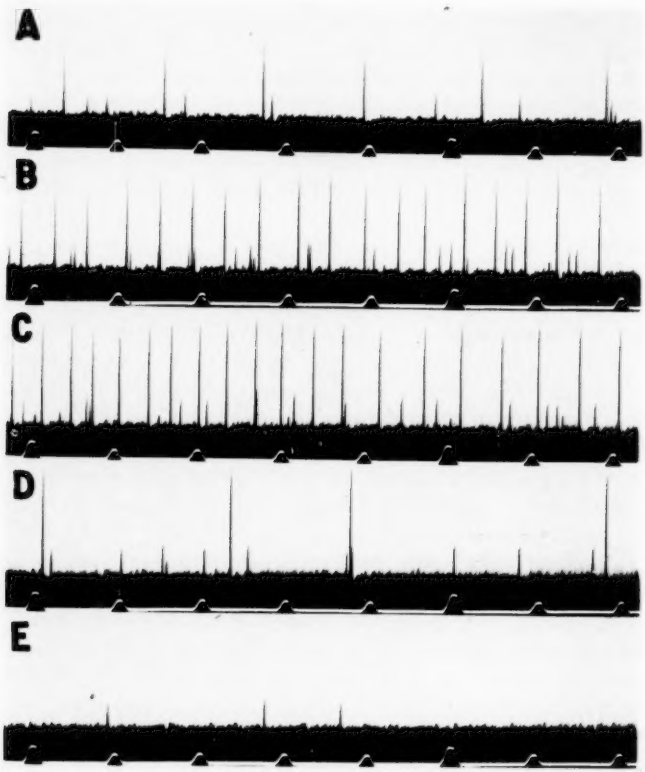


Fig. 5. The discharge of impulses in two fibers of the cervical sympathetic nerve in response to stimulation of the hypothalamus at succeeding dorso-ventral levels separated by 1 mm.

final preganglionic neurone located in the anterolateral column of the cord. An increase in intensity of hypothalamic stimulation increases the number of these pathways in action. A shift in the position of the stimulating electrode may bring a greater number of these pathways within the zone of influence of the stimulus, and thus increase the number in action. The discharge frequency of the final common neurone is, among other things,

some function of the number of parallel pathways in action. These experiments shed no light on the anatomical makeup of these pathways, i.e., upon the number of synapses between hypothalamus and final common path; but that there is extensive decussation within the system is evident.

C. *Stimulus frequency.* The effect of increasing the frequency of hypothalamic stimulation upon the discharge in the inferior cardiac nerve and upon the associated rise in blood pressure is similar to that of increasing the intensity of stimulation. In fact, records identical in all respects to

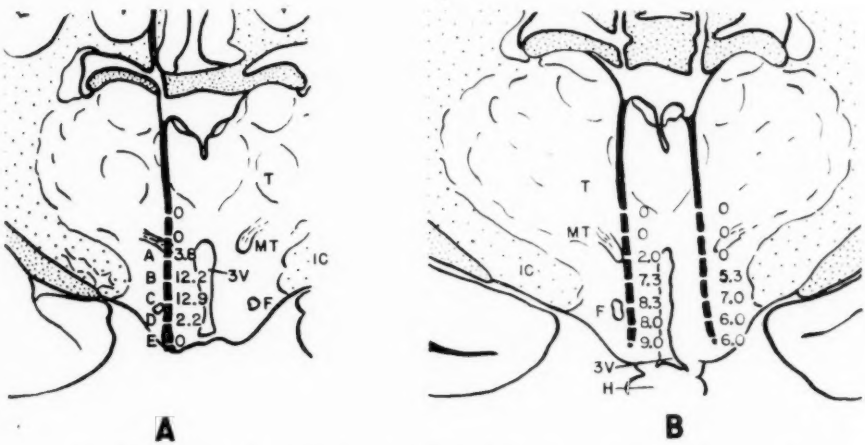


Fig. 6A. Tracing of a stained section through the diencephalon of the cat. The heavy vertical line indicates the track of the stimulating electrodes; the interruptions of the line the position of the bare electrode tips. Letters A to E on the left correspond to the points which were stimulated during preparation of the similarly lettered records of figure 5. The numbers to the right of the electrode track give the frequency of discharge in impulses per second of the cervical sympathetic neurone of large spike potential.

B. Location of stimulating electrodes in a similar experiment in which a single cervical sympathetic neurone was caused to discharge at the indicated frequencies by stimulation at a number of levels on both sides of the hypothalamus.

those shown in A, B and C of figure 2 have been obtained by stimulation of the hypothalamus with frequencies of 50, 100 and 150 per second, maintaining the intensity constant. The degree of activity in the cardiac nerve during hypothalamic stimulation and the duration of the period of inhibition of spontaneous activity after stimulation increase in proportion to the frequency of stimulation. Likewise, the magnitude of the rise in blood pressure and the length of time that it remains elevated show a similar proportionality to frequency of stimulation.

The response of single fibers of the cervical sympathetic to an increase

in frequency of hypothalamic stimulation is qualitatively similar to that shown in figure 3 for an increase in stimulus intensity. Quantitatively, however, the results differ. In the same experiment from which figures 3 and 4 were taken, the stimulus intensity was maintained constant and the frequency varied. A plot of the response frequency of the single neurone against the stimulus frequency applied to the hypothalamus yields a relationship which is remarkably linear, figure 7. The frequency of firing of the neurone in this experiment maintained an essentially constant ratio to the frequency of hypothalamic stimulation, the neurone firing once to approximately every twenty-fifth stimulus throughout the range investigated.

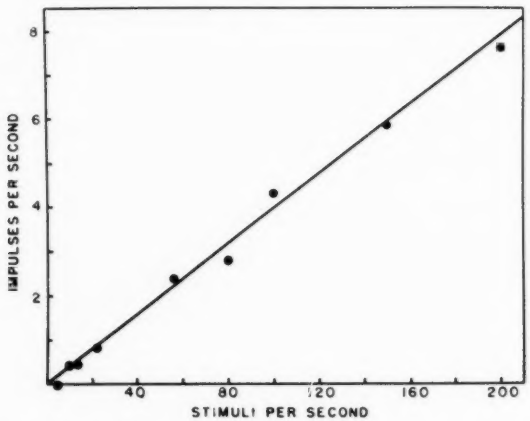


Fig. 7. The relation between frequency of discharge of impulses in a single fiber of the cervical sympathetic nerve and frequency of hypothalamic stimulation. Intensity of stimulation constant.

Not only does an increase in stimulus frequency increase the activity of any single unit, but it also brings into activity new units, not active at the lower stimulus frequency. Figure 8 illustrates the effect of increasing the frequency of hypothalamic stimulation on the number of neurones responding in a multifiber preparation. In records A, B and C the stimulus frequencies were 8.5, 18 and 43, respectively, the intensity being maintained constant. In records A and B only the neurone characterized by the high, thin spike potential responded. In record C, at the higher frequency of stimulation, the response of this same neurone may again be identified, but a number of other neurones whose spike potentials are different in form also responded. Facilitation at some point in the descending pathways from the hypothalamus probably accounts for this radiation of excitation over channels not responsive to volleys of lower frequency.

The results presented above indicate not only that multiple pathways descending from the hypothalamus converge on single effector neurones but also that each descending pathway establishes connections which diverge and impinge on many effector neurones. Experiments presented in succeeding pages indicate that important loci of convergence and divergence lie in the medullary sympathetic centers.

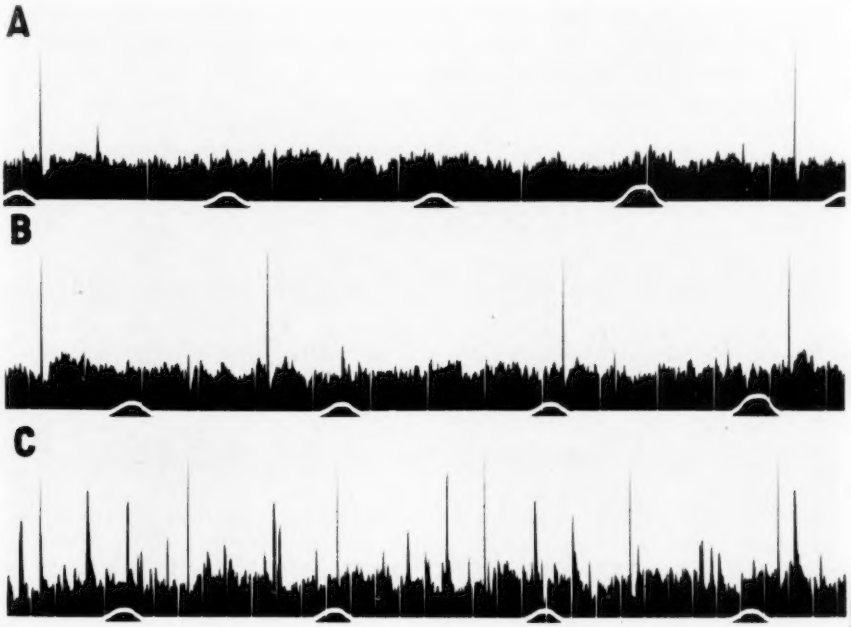


Fig. 8. Discharge of impulses in a multifiber preparation of the cervical sympathetic nerve during stimulation of the hypothalamus at frequencies of 8.5, 18 and 43 shocks per second. In record C at the higher rate of stimulation several fibers respond in addition to the one active in A and B.

D. *Reversal of response from change in frequency of stimulation.* The responses to hypothalamic stimulation which have been described in the preceding paragraphs, all support the generally accepted view that the hypothalamus serves as a center for the regulation of sympathetic function. Cushing (1931) first ascribed importance to the hypothalamus as a regulating center for parasympathetic functions as well. Beattie (1932) and Fulton (1932) maintained that the hypothalamus could be divided functionally into an anterior parasympathetic portion and a posterior sympathetic portion. Subsequent work reviewed by Ranson and Magoun

(1939) and Ingram (1939) as well as our own has given little support to such a view. We have found no difference in the type of sympathetic response on stimulation of the supraoptic, tuberal or mammillary portions of the hypothalamus although the lateral and more caudal parts seem to be somewhat more reactive. We have not, however, studied parasympathetic activity.

An interesting reversal from a pressor to a depressor type of response has been observed occasionally on stimulation of the hypothalamus at low frequencies. The records presented in figure 9 A and B are typical of such an experiment. Record A shows the usual pressor response and the increased activity in the inferior cardiac nerve from low intensity

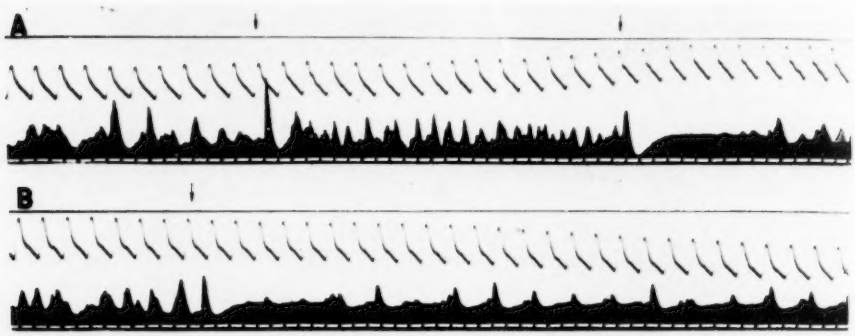


Fig. 9. Reversal from a pressor to a depressor response on lowering the frequency of hypothalamic stimulation. A. Stimulation of the hypothalamus at 20 per second; sympathetic excitation and rise of blood pressure. B. Stimulation at 2 per second; sympathetic inhibition and fall of blood pressure. Arrows indicate beginning of stimulation and also the end in A. Time, $\frac{1}{5}$ second.

hypothalamic stimulation at a frequency of 20 per second. In record B the intensity of stimulation and the placement of the electrodes were unchanged but the frequency of stimulation was reduced to 2 per second. Stimulation at this lower frequency led to a partial inhibition of spontaneous activity in the cardiac nerve and a gradual fall in blood pressure. Both the slight slowing of the heart (5 per cent) and the fall in blood pressure were unchanged by section of the vagi.

Although this reversal from a pressor to a depressor response has been noted in several experiments, we have not been able to repeat it at will. There seems to be no characteristic part of the hypothalamus from which this reaction may be elicited. Hare and Geohegan (1939) and Berry and Hodes (1941) have noted this same reversal on lowering the frequency of hypothalamic stimulation.

There is a noteworthy similarity between the response reversal from hypothalamic and from peripheral nerve stimulation. In both, the lower frequencies favor the depressor type of response, the higher frequencies the pressor type. Whether this response reversal results from activation of two fiber systems in the hypothalamus as has been postulated for peripheral nerve (Ranson, 1916), or is an expression of the effects of volleys of impulses of different frequencies over the same pathways is unknown. Kabat *et al.* (1935) maintain that depressor pathways originating in the preoptic and more cephalic regions of the forebrain descend through the hypothalamus. It is possible that such pathways are more easily excited by low frequency stimulation than are the pressor ones, though we have no evidence on this matter. These experiments suggest, however, that some of the so-called parasympathetic responses which have been described as resulting from hypothalamic stimulation actually result from inhibition of tonic sympathetic outflow.

Relation between frequency and intensity of hypothalamic stimulation. Since a change of position of the stimulating electrodes within the hypothalamus may alter the number of active projections which impinge upon any given sympathetic neurone, it is unlikely that any two neurones in the same or different preparations will ever behave identically to a given stimulus intensity or frequency. The maximum frequency of discharge of sympathetic neurones, at the highest intensity and frequency of hypothalamic stimulation which can be applied without causing any considerable degree of somatic motor response, varies between 10 and 50 impulses per second. A part of this variation is undoubtedly due to differing numbers of pathways to that neurone which lie within the zone of influence of the stimulus. Another factor in this variation may be differences in excitability of the several neurones. Although there are these marked differences in the quantitative behavior of sympathetic neurones, the qualitative aspects of their behavior in relation to changes of frequency and intensity of hypothalamic stimulation are remarkably reproducible.

In 7 experiments in which the relationship between impulse frequency and stimulus intensity was examined, it was found to have the characteristic sigmoid form of figure 4, although the maximum impulse frequency varied between 10 and 50. Similarly in 8 experiments a relationship was noted between impulse frequency and stimulus frequency which was linear over a considerable part of the range, though here again the peak frequencies varied over wide limits.

If it is true that the peak frequencies of impulse initiation in these experiments are related to the number of pathways excited as well as the frequency of their excitation, then an increase in intensity of stimulation should not alter the general character of the relationship between impulse frequency and stimulus frequency, but merely change magnitudes. In figure 10,

records A, B, C and D, E, F, the same three frequencies of stimulation were applied to the hypothalamus. In the A to C group, the relative intensity was 1; in the D to F group, it was 3. That an increase in intensity at each

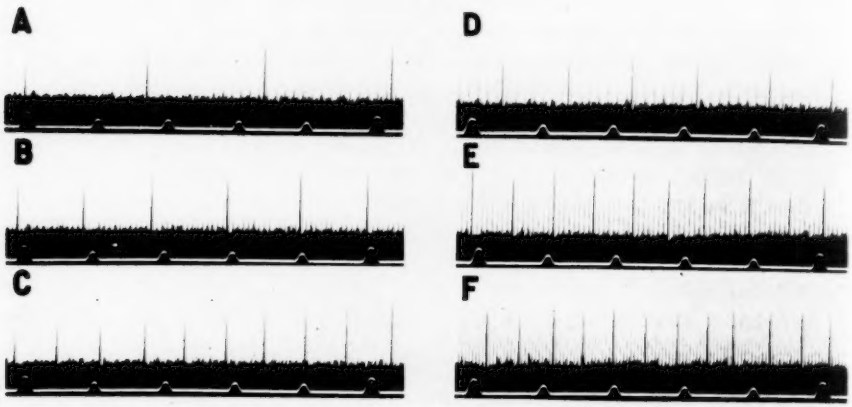


Fig. 10. The discharge of impulses in a single fiber of the cervical sympathetic nerve during hypothalamic stimulation at 3 frequencies and 2 intensities. Left column, A to C, intensity of 1; right column, D to F, intensity of 3. Frequency of upper row, 38; middle row, 68; lower row, 100 per second. Time, $\frac{1}{5}$ second.

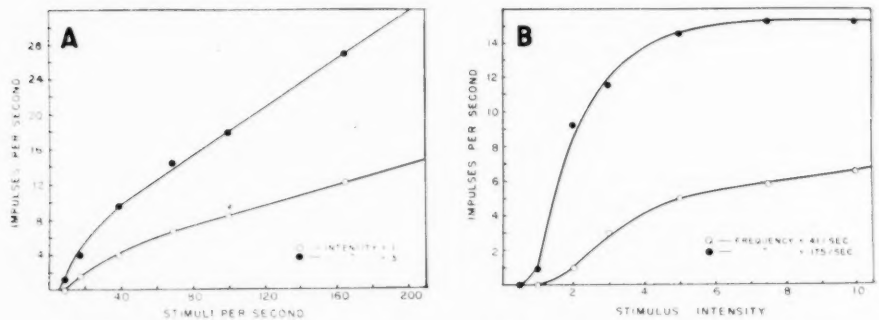


Fig. 11A. The relation between frequency of discharge of impulses in a single fiber of the cervical sympathetic nerve and frequency of hypothalamic stimulation at relative intensities of 1 and 3.

B. Similar relationship between frequency of discharge and intensity of stimulation at stimulus frequencies of 41 and 175 per second.

frequency of stimulation produces an increase in impulse frequency is apparent by comparing A, B, C (low intensity), with D, E, F (higher intensity). In figure 11 A the complete data of such an experiment are

presented graphically. The relation between impulse frequency and stimulus frequency at each intensity is linear throughout the greater part of the range, though the slopes differ. The peak frequency of this neurone varies between 12 and 27, depending on the numbers of pathways excited, yet the general relationship is unchanged.

One difference in this relationship which we have noted between different neurones is illustrated by comparing figures 7 and 11 A. An extrapolation of the linear part of the relation in figure 7 passes through the origin; in figure 11 A it strikes above the origin. It seems plausible to assume that while the two neurones do not fire spontaneously, the one whose behavior is illustrated by figure 10 A may be receiving subliminal excitation from another source than the hypothalamus, while the neurone of figure 7 does not. Such subliminal excitation summing with hypothalamic excitation might produce a frequency of firing at each stimulus frequency higher by a constant amount than would be predicted.

As illustrated by figure 11 B, a change of stimulus frequency from 41 per second to 175 per second does not seriously alter the form of the relationship between impulse frequency and stimulus intensity, although the peak values vary between 7 and 15 impulses per second as a result of increasing the rate of stimulation of a constant number of pathways.

Integration of hypothalamic cardiovascular responses. A. *Antagonism between buffer reflexes and hypothalamic cardiovascular responses.* Stimulation of the hypothalamus with even low intensity stimuli disturbs to a considerable degree the delicate regulation which maintains blood pressure and heart rate within normal limits. Some aberration of the normal is prerequisite for the hypothalamus to exercise its function of adjustment to emotional and thermal factors in the environment, yet the basic need for regulation remains unchanged. Since hypothalamic activity and activity of the buffer reflexes are in direct opposition to one another, the degree of their interplay is an important factor in analyzing cardiovascular regulation.

Bronk and Stella (1932) have shown that pressure sensitive endings within the walls of the carotid sinus and aorta discharge impulses whose frequency is proportional to the pressure. A rise in pressure increases the frequency of discharge and brings into activity new endings which did not respond at the lower pressures. These impulses carried to the medullary cardiovascular centers over the nerves of Hering and the depressor nerves produce an inhibition of tonic sympathetic outflow to the heart and blood vessels which results in a lowering of pressure toward normal (Bronk, Ferguson and Solandt, 1934).

Evidence of the activity of the buffer mechanism is seen in figure 1. The tonic activity in the inferior cardiac nerve, which is evident at normal pressure levels prior to hypothalamic stimulation, is completely abolished

during the period of elevated pressure following the cessation of stimulation. Records from single fibers of the depressor nerve clearly indicate that the pressure sensitive endings within the aortic arch respond to the rise in blood pressure produced by hypothalamic stimulation with an increasing frequency of discharge. Central stimulation of the depressor nerve at a comparable frequency causes inhibition of the tonic sympathetic outflow over the cardiac nerve.

Such experiments indicate the extent to which the buffer reflexes operate in returning conditions to normal following hypothalamic stimulation. Experiments of another type, however, must be performed to show whether the buffer reflexes act to oppose the changes induced during the period of hypothalamic stimulation. If brief bursts of high frequency stimuli are applied to the hypothalamus at intervals of 1 second, each burst of stimuli is

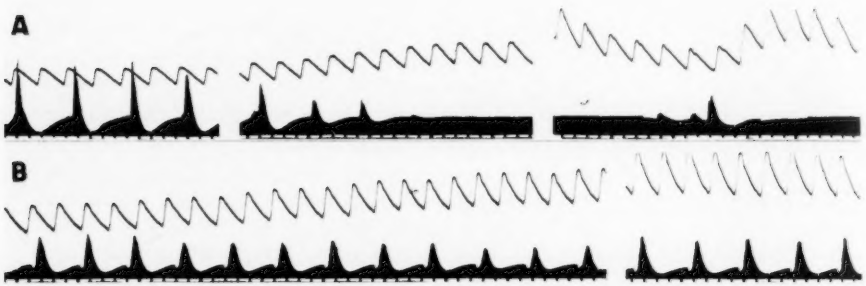


Fig. 12. Inhibition of sympathetic activity in the inferior cardiac nerve by the elevation of blood pressure following the injection of adrenalin. A, low intensity stimuli to the hypothalamus; B, high intensity stimuli. Time, $\frac{1}{5}$ second.

followed by a synchronized volley of impulses in the inferior cardiac nerve. Roughly, the height of the volley is indicative of the number of sympathetic neurones responding. In the experiment from which figure 12 A was taken, bursts of stimuli of low intensity were applied to the hypothalamus once a second throughout the entire record. Adrenalin was injected during the interval indicated by the first break in the record. As the blood pressure rose, the volleys decreased progressively in height, until inhibition became complete, although stimulation continued unchanged. In the last part of the record, a fortuitous fall in blood pressure permitted a volley to escape, indicating most clearly that inhibition of the periodic hypothalamic drive resulted from the rise in pressure. The experiment was then repeated in an identical manner except that the intensity of the bursts of hypothalamic stimulation was increased. Record B illustrates that the buffer reflexes, brought into play by the rise in blood pressure could not completely inhibit

the volleys initiated by the more intense hypothalamic stimuli, though they could reduce for a time the number of neurones responding.

An extension of this principle is illustrated by the records of figure 13. A single neurone of the cervical sympathetic was caused to fire repetitively by high frequency, very low intensity stimulation of the hypothalamus,

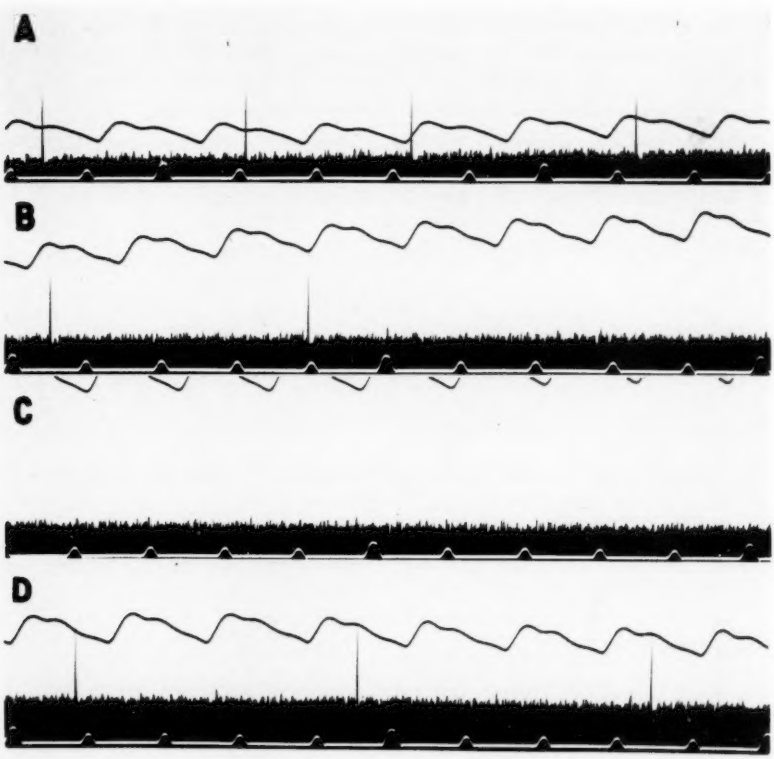


Fig. 13. Slowing and inhibition of discharge in a single fiber of the cervical sympathetic nerve by the elevation of blood pressure following the injection of adrenalin. Between records A and B, adrenalin was administered intravenously. Time, $\frac{1}{5}$ second.

maintained constant throughout the entire series of records. In the interval between records A and B, adrenalin was given intravenously, and with the rise in pressure, the response frequency diminished until the neurone ceased to fire, record C. With return of pressure toward normal, the neurone again began to fire repetitively, record D. Stimulation of the hypothalamus with more intense stimuli caused this neurone to fire

more rapidly, and under these circumstances, a rise of blood pressure produced a negligible slowing of the response.

These experiments indicate conclusively that the buffer reflexes do act to oppose a rise of pressure during the period of hypothalamic stimulation. Furthermore, it is clear that activation of the buffer reflexes interposes a relative block between hypothalamus and efferent sympathetic outflow. If the buffer reflexes are activated maximally, and if the intensity of hypothalamic stimulation is low, the block can be an absolute one. Or it may be only a relative block, diminishing the number of responding efferent motor neurones and the frequency at which they respond if the intensity of hypothalamic stimulation is high. Such results can best be interpreted on the basis of a synaptic break in the descending hypothalamic pathways, at which the afferent buffer nerves may exert an inhibitory action.

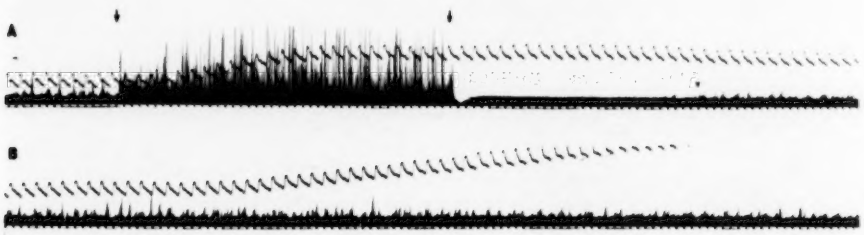


Fig. 14A. Increased discharge in the inferior cardiac nerve during hypothalamic stimulation and inhibition following stimulation in an animal in which the buffer nerves had been sectioned bilaterally. Arrows indicate beginning and end of stimulation.

B. Minimal inhibition of spontaneous discharge during the elevation of blood pressure produced by the injection of adrenalin in the same animal. Time, $\frac{1}{5}$ second.

A factor, other than buffer reflex activation, also operates to produce the inhibition of spontaneous sympathetic activity which follows hypothalamic stimulation. The operation of this factor is clearly illustrated by hypothalamic stimulation in an animal in which the buffer nerves have been sectioned. Figure 14 shows that the inhibition of spontaneous activity following hypothalamic stimulation is marked (record A) even though the carotid sinus and depressor nerves had been sectioned bilaterally. The completeness of removal of the pressure sensitive afferents is illustrated by record B. The administration of intravenous adrenalin produced a considerable rise in blood pressure, yet there was only a minimal degree of inhibition of spontaneous sympathetic activity. The inhibition following hypothalamic stimulation in this experiment cannot be due to the rise in blood pressure. It appears to result from a diminished excitability of

the sympathetic centers following intense activity. An analysis of the time course of these excitability changes is the subject of another communication.

B. *Synergism between buffer reflexes and hypothalamic cardiovascular responses.* The tonic discharge of impulses in the inferior cardiac nerve commonly shows a marked pulse modulation. This synchronization of sympathetic volleys with the pulse depends upon the integrity of the buffer nerves, for when these nerves are sectioned, the volleys occur at random and more rapid frequencies. Figures 1 and 2 show that stimulation of the hypothalamus with high frequency stimuli of moderate intensity causes the discharge of impulses in volleys of random and relatively high frequency. The buffer nerves obviously are exerting no very marked control over the increased sympathetic activity induced by such intensities of stimulation. If, however, the hypothalamus is stimulated at low intensity with high frequency shocks, the moderately increased sympathetic activity

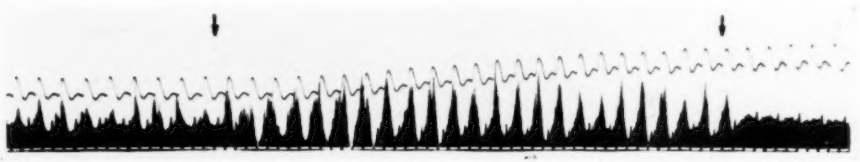


Fig. 15. Groups of sympathetic impulses in the cardiac nerve synchronous with the pulse. Increase of this grouped discharge during stimulation of the hypothalamus at 100 per second. Arrows indicate beginning and end of stimulation. Time, $\frac{1}{5}$ second.

may be shown to be checked and controlled by the buffer reflexes much as is the normal spontaneous activity. Figure 15 illustrates such synergistic action between the buffer reflexes and activity induced by low intensity hypothalamic stimulation. The first part of the record illustrates the spontaneous tonic sympathetic discharge synchronous with the pulse. During the interval marked off by the arrows, low intensity high frequency stimuli were applied to the hypothalamus. Stimulation results in both a rise in blood pressure and an increase in sympathetic activity in the cardiac nerve. But most striking is the fact that this activity is an accentuation of the normal, showing an even more definite pulse modulation. The conclusion is inescapable that such moderate increases in sympathetic activity induced by hypothalamic stimulation are woven into the normal pattern of sympathetic cardiovascular response without serious disarrangement of regulation.

C. *Summation of hypothalamic and peripheral nerve pressor responses.* The pressor response obtained on central stimulation of a peripheral sensory nerve is familiar to every student of physiology. That it may be

obtained in the decerebrate animal and is independent of structures above the pons for its central integration is similarly common knowledge. The central course of the pressor reflex pathway in the tract of Lissauer, integration at a medullary level, and efferent outflow through the ventrolateral columns of the cord were demonstrated by Ranson (1916).

We have observed on stimulation of a sensory nerve an increase in activity in the cardiac nerve and a rise in blood pressure which is qualitatively similar to that observed on hypothalamic stimulation. Thus central stimulation of the sciatic, femoral or peroneal nerves causes an abrupt increase in activity in the cardiac nerve, a rise in blood pressure after a latency of more than second, and following stimulation, an inhibition of spontaneous discharge in the nerve which lasts for the duration of the elevated blood pressure. The differences between the peripheral nerve pressor response and the response to hypothalamic stimulation are chiefly quantitative. In our experience the pressor response from nerve stimulation is variable in the anesthetized animal, unobtainable in some, and always less in magnitude than that obtained on hypothalamic stimulation.

The results obtained, however, by simultaneous stimulation of the hypothalamus and a peripheral sensory nerve emphasize the essential similarity between the pressor responses elicited from the two sources. If strengths of stimuli are so adjusted as to produce comparable responses from hypothalamic and nerve stimulation, simultaneous stimulation leads to a response qualitatively identical to that from the two sources singly, but of a magnitude equivalent to their sum.

The foregoing observations indicate that hypothalamic activity is not permitted, unchecked, to disturb the balance of cardiovascular regulation. Nor, on the other hand, is buffer reflex adjustment so inflexible, that deviations of large magnitude are impossible. Rather, these experiments suggest that there is operative a delicate system of checks and balances which permits a deviation from normal directly related to the intensity of hypothalamic activity and inversely related to the degree of disturbance caused thereby. The buffer reflexes exert a control over sympathetic outflow induced by hypothalamic activity much as they do over spontaneous activity originating within the medullary centers. If the increase in sympathetic activity is slight, it is modulated and fitted into the same pattern as is the normal. If the increase is great, the buffer reflexes, no longer able to modulate it, at least hold it in check by a relative block at the medullary level. This block may operate to diminish both the number of sympathetic neurones responding and the frequency of response of any given neurone. The essential similarity between the pressor response from hypothalamic and sensory nerve stimulation, and the demonstration that they are capable of summation, suggests that under certain circumstances the two may show mutual re-enforcement. Such re-enforcement might

prove of value in activities where there is association of painful stimuli with the affective state.

Characteristics of the excitatory process. The factors so far considered as determining the response of sympathetic neurones to hypothalamic stimulation have been concerned with the more gross overall excitability relationships. However, if one examines certain experiments more closely, details of relationships appear. A single stimulus applied to the hypo-

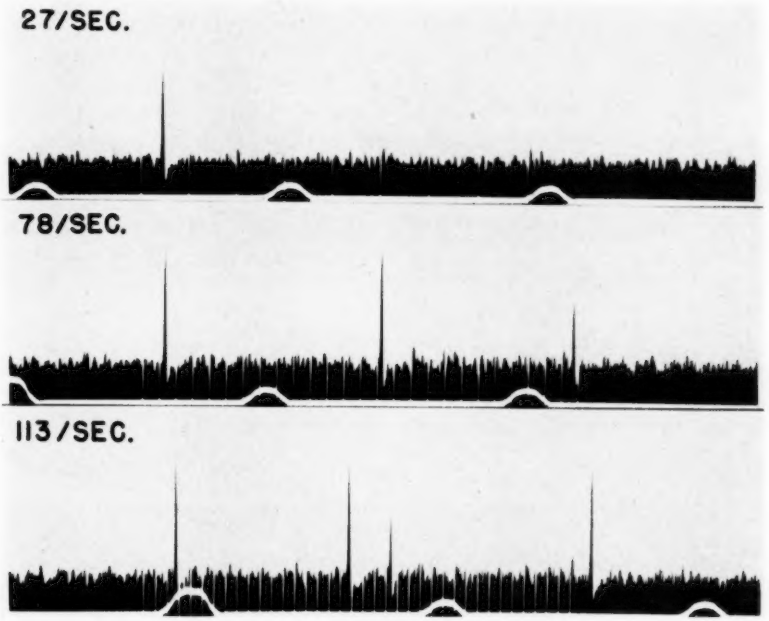


Fig. 16. Discharge of impulses in two fibers of the cervical sympathetic nerve in response to stimulation of the hypothalamus at the indicated frequencies. Time, $\frac{1}{5}$ second.

thalamus sets up a single volley of descending impulses whose magnitude, i.e., the number of participating pathways, depends at any one placement of the electrodes upon the stimulus strength. Such a volley of impulses may or may not cause a particular neurone of the cervical sympathetic to discharge an impulse. Most neurones which we have studied do not respond to single volleys of ordinary intensity, though some do. The response of both types of neurones to repetitive volleys is illustrated by figure 16. The neurone characterized by the larger spike potential fires in response to a single volley. The neurone characterized by the smaller

spike potential fires only after the summated effects of some 20 or more volleys. That there is no fundamental difference between these two neurone types is indicated by the fact that a neurone not responding to a single volley may be caused to do so by introducing into the hypothalamus high frequency subliminal stimuli through a second pair of electrodes.

Figure 3 illustrates a second principle, namely, the number of volleys which must be summated to fire a particular neurone depends upon the stimulus strength. In record A of figure 3, 0.43 second of hypothalamic stimulation at a frequency of 100 volleys per second was required before

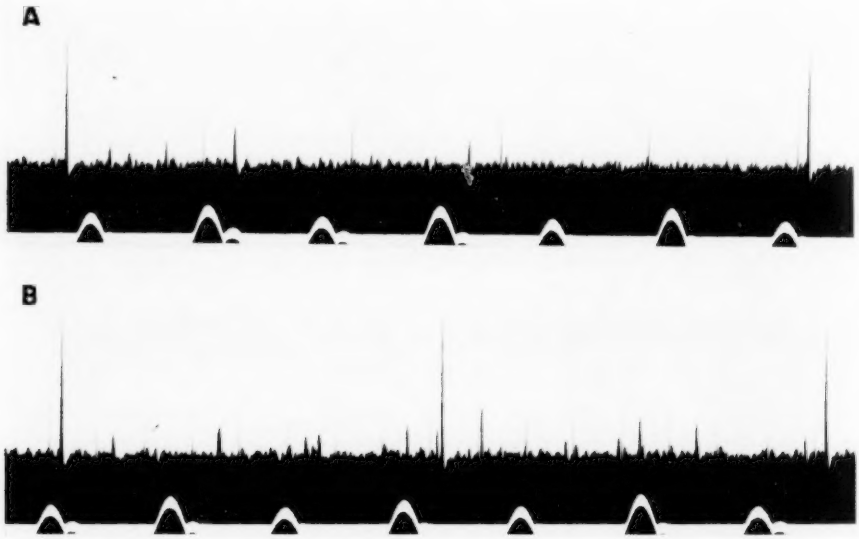


Fig. 17. Discharge of impulses in a single fiber of the cervical sympathetic nerve in response to stimulation of the hypothalamus at 5 and 13 per second. The stimuli are indicated by the faint upward deflections at regular intervals throughout each record. An impulse appears after the first and last stimuli in A. Three impulses are recorded in B. Time, $\frac{1}{5}$ second.

the neurone gave its initial response. In record B an increase of 35 per cent in the intensity of the stimulus reduced the time to the first response to 0.03 second.

Figure 17 illustrates a third principle, namely, that independent of the number of volleys which must summate before a neurone fires, the neurone fires in response to or is triggered by a particular volley. There is a relatively constant latency in this experiment of 18 to 22 msec. from the start of a particular volley of impulses in the hypothalamus, to the arrival of a particular impulse in the cervical sympathetic at the point of recording in the neck.

Figures 3 and 16 also illustrate a further point of interest. The time from the beginning of stimulation to the first impulse is always less than the time from the first to the second impulse, often considerably less. Furthermore, the intervals between succeeding impulses tend to lengthen. In consequence of these changes, the neurone fires most rapidly at the beginning of stimulation, adapts to a lower frequency over the first second, and then maintains a frequency which only slowly declines over the remaining period of stimulation.

The observations just cited may be qualitatively described in terms used in studies on spinal reflexes (Creed, Denny-Brown, Eccles, Liddell, Sherrington, 1934) and on ganglionic transmission (Eccles, 1937), though it must be emphasized that our use of the terminology implies nothing as to the fundamental mechanisms involved. Thus the initial firing of the sympathetic neurone in response to hypothalamic stimulation depends on the building up of a central excitatory state by repetitive volleys from the hypothalamus, to a critical level, at which a single volley (detonator) triggers the mechanism and fires the neurone. Once the neurone has fired, the subsequent behavior of the system depends in part upon the average level of excitatory state maintained by the hypothalamic volleys, and in part upon the time course of recovery of excitability, a subject to be treated in another communication. In fact the steady state, which is characterized by repetitive firing of the cervical sympathetic neurone at a constant frequency to maintained hypothalamic stimulation, may be most readily visualized as a balance between these two factors.

The rate at which the excitatory state is initially built up and the final average level it maintains are directly related to the frequency of the hypothalamic volleys, and the number of pathways carrying volleys. The time for recovery of excitability, however, is directly related to the frequency at which the system fires. It follows that the increased frequency of firing resulting from increase in frequency or intensity of hypothalamic stimulation is attained by maintaining a higher average level of central excitation capable of exciting earlier in the recovery cycle. The period of rapid decline in discharge frequency at the start of stimulation finds explanation in summation of subnormality requiring a progressively increasing recovery time, which after a second or less becomes stabilized.

If we attempt to pass from the general to the specific in explanation of the above phenomena, no one incontrovertible fact enables us to choose between several current theories. The excitatory state which in our experiments shows such definite temporal summation can logically be explained in terms of relatively long persistence at synapses of the excitatory changes produced by single volleys (Dale, 1937; Eccles, 1937; Barron and Matthews, 1938; Bronk, 1939). However, the unknown, but obviously complex, configuration of the tegmental projections from the hypothalamus allows

for the setting up of complicated chains of interneurones wherein synaptic changes of brief duration could account for the relations noted (Lorente de Nó, 1939).

SUMMARY

Stimulation of the hypothalamus of the anesthetized cat with brief repetitive condenser shocks of moderate intensity leads, after a latency of less than 0.1 second, to an abrupt increase in activity of sympathetic nerves to the heart and blood vessels. This activity ceases equally abruptly when stimulation is stopped, and for a variable period thereafter all spontaneous activity in these nerves is inhibited. There is no evidence under the conditions of our experiments that any sympathetic after discharge results from hypothalamic stimulation. Blood pressure begins to rise some 1 to 2 seconds after the start of hypothalamic stimulation, and may continue to rise and remain elevated several seconds after stimulation is stopped. The delay in the rise of blood pressure and the prolongation of the rise result from latency and inertia of the sympathetic effector, not from any corresponding delay or persistence of neural activity.

An increase in intensity or frequency of hypothalamic stimulation increases the magnitude and duration of the rise in blood pressure. This increased effector response is brought about by an increase in the number of sympathetic motor neurones set into activity and by an increase in the frequency of response of each neurone.

Multiple pathways descend from both sides of the hypothalamus to make connection with each sympathetic motor neurone. The frequency of response of the neurone is a function of the number of these pathways excited and of the frequency at which they are excited.

While stimulation of the lateral and posterior portions of the hypothalamus yields responses of greater magnitude, no qualitative differences have been noted on stimulation of the preoptic, tuberal or mammillary divisions.

The buffer reflexes which control the spontaneous sympathetic outflow from the medullary centers, also moderate the outflow induced by hypothalamic stimulation. Activation of the buffer afferents may inhibit all response of sympathetic motor neurones to hypothalamic stimulation or reduce the number of these neurones responding. Similarly the frequency of response of any single neurone may be reduced or the response entirely abolished by activation of the buffer afferents. The buffer afferents impress a pulse modulation upon mild increases of sympathetic activity which result from hypothalamic stimulation in exactly the same way that they modulate spontaneous sympathetic outflow. These facts are interpreted as indicating that sympathetic responses from hypothalamic stimulation are mediated through medullary sympathetic centers, not by direct connection of descending hypothalamic pathways with sympathetic motor neurones.

The frequency of firing of a sympathetic motor neurone in response to hypothalamic stimulation is determined by the level of excitation maintained by the hypothalamic volleys, the time course of the recovery cycle, and the degree of activity of inhibitory afferents at some critical point between hypothalamus and motor neurone.

REFERENCES

ADRIAN, E. D. AND D. W. BRONK. J. Physiol. **66:** 81, 1928.

BARD, P. This Journal **84:** 490, 1928.

BARRON, D. H. AND B. H. C. MATTHEWS. J. Physiol. **92:** 276, 1938.

BEATTIE, J. Canad. M. A. J. **26:** 400, 1932.

BERRY, C. AND R. HODES. This Journal **133:** P25, 1941.

BRONK, D. W. Harvey Lect. **29:** 245, 1934.
J. Neurophysiol. **2:** 380, 1939.

BRONK, D. W. AND L. K. FERGUSON. This Journal **110:** 700, 1935.

BRONK, D. W., L. K. FERGUSON AND D. Y. SOLANDT. Proc. Soc. Exper. Biol. and Med. **31:** 579, 1934.

BRONK, D. W., L. K. FERGUSON, R. MARGARIA AND D. Y. SOLANDT. This Journal **117:** 237, 1936.

BRONK, D. W., R. F. PITTS AND M. G. LARRABEE. Res. Pub. Assoc. Nerv. Ment. Dis. **20:** 323, 1940.

BRONK, D. W. AND G. STELLA. J. Cell. and Comp. Physiol. **1:** 113, 1932.

CREED, R. S., D. DENNY-BROWN, J. C. ECCLES, E. G. T. LIDDELL AND C. S. SHERRINGTON. Reflex activity of the spinal cord. The Clarendon Press, Oxford, 1932.

CUSHING, H. Proc. Nat. Acad. Sc. **17:** 163, 239, 1931.

DALE, H. Harvey Lect. **32:** 229, 1937.

Eccles, J. C. Physiol. Rev. **17:** 538, 1937.

ECTORS, L., N. L. BROOKENS AND R. W. GERARD. Arch. Neurol. and Psychiat. **39:** 789, 1938.

FULTON, J. F. New England J. Med. **207:** 60, 1932.

GRINKER, R. R. AND H. J. SEROTA. J. Neurophysiol. **1:** 573, 1938.

HARE, K. AND W. A. GEOHEGAN. This Journal **126:** P524, 1939.

HOUSSAY, B. A. AND E. A. MOLINELLI. Compt. rend. Soc. de biol. **93:** 1454, 1925.

INGRAM, W. R. Psychosom. Med. **1:** 48, 1939.

KABAT, H., H. W. MAGOUN AND S. W. RANSON. Arch. Neurol. and Psychiat. **34:** 931, 1935.

KARPLUS, J. P. AND A. KREIDL. Pflüger's Arch. **219:** 613, 1928.

LORENTE DE NÓ, R. J. Neurophysiol. **2:** 402, 1939.

MAGOUN, H. W. This Journal **122:** 530, 1938.

MAGOUN, H. W., S. W. RANSON AND A. HETHERINGTON. This Journal **119:** 615, 1937. Arch. Neurol. and Psychiat. **39:** 1127, 1938.

MORRISON, R. S. AND D. M. RIOCH. This Journal **120:** 257, 1937.

PITTS, R. F. This Journal, in press.

RANSON, S. W. This Journal **42:** 1, 1916.

RANSON, S. W. AND H. W. MAGOUN. Ergebn. d. Physiol. **41:** 56, 1939.

WANG, S. C. AND S. W. RANSON. J. Comp. Neurol. **71:** 437, 1939.

FORMATION OF THE R COMPLEX OF THE ELECTROCARDIOGRAM[1]

L. H. NAHUM, H. E. HOFF AND W. KAUFMAN[2]

From the Laboratory of Physiology, Yale University School of Medicine, New Haven, Conn.

Accepted for publication June 25, 1941

In a previous study a method was described which permitted the separate registration of what appeared to be the contribution of the right and left ventricles to the electrocardiogram (1). It was concluded that the normal electrocardiogram is formed by the algebraic summation of these components, i.e., the dextro- and levocardiograms. The dextrocardiogram, a monophasic-like complex directed upward, was found to precede by a short interval the levocardiogram, a similar complex of opposite polarity. This asynchrony in excitation of the two ventricles has been noted before (2). A portion of the upstroke of the dextrocardiogram is therefore able to develop without opposition from the levocardiogram. This unopposed dextrocardiogram is recorded in the electrocardiogram as the upstroke of R.[3] The onset of the levocardiogram, which is of opposite polarity to the dextrocardiogram, arrests the further ascent of the dextrocardiogram and gives rise to the downstroke of R (fig. 1). The summit of the R complex therefore marks the moment when the major part of the surface of the left ventricle becomes electrically active, and the interval between the base and the summit of R (i.e., from the end of the downstroke of Q to the summit of R) represents the interval separating the onset of the dextro- and levocardiograms.

The amplitude of the R complex does not therefore necessarily indicate the height of the dextrocardiogram, since the levocardiogram may start before the full development of the dextrocardiogram. Certain evidence supports this view: 1, dextrocardiograms are seen occasionally which are higher than the R complex of the original electrocardiogram; 2, T waves

[1] Aided by grants from Fluid Research Funds, Yale University and Emanuel Libman Fellowship Fund.

[2] Fellow of the Dazian Foundation.

[3] The Q wave is not discussed for purposes of simplification. When Q is present in the electrocardiogram, the factors which are responsible for the R complex cannot be considered to be the primary events. The nature of Q and its relation to R will be considered in a later communication.

may be found with an amplitude exceeding R (3, fig. 2), and 3, the initial deflection of certain ventricular extrasystoles is often greater than the amplitude of the R wave in the normal complex.

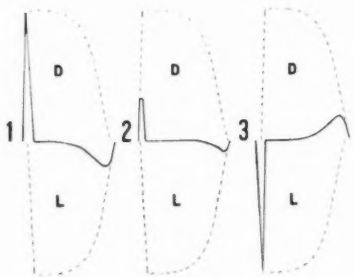


Fig. 1

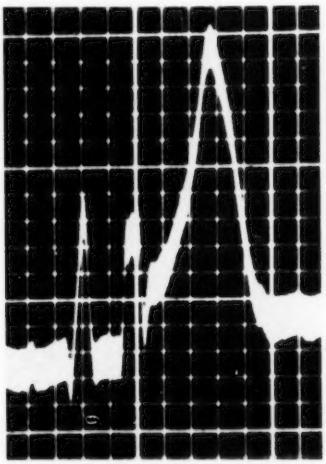


Fig. 1A

Fig. 1. A hypothetical diagram illustrating the formation of the R wave. In 1 the levocardiogram, *L*, begins at the instant the dextrocardiogram, *D*, reaches its full height. The resulting R complex (shown in heavy lines) is upright, and equals the dextrocardiogram in amplitude. In 2 the levocardiogram is initiated relatively sooner than in 1, and the R complex which results from this summation is lower, and its width is reduced. In 3 the levocardiogram precedes the dextrocardiogram and in this case the resulting R is directed downward.

It is obvious that in 2 a small plateau will develop at the apex of R if the dextro- and levocardiograms develop at equal velocities along a straight line. Such plateaus were in fact seen in several experiments (fig. 1a). In others (e.g., fig. 2c) no such plateau was seen despite extreme reduction in the amplitude of R. The absence of a plateau could be explained by postulating that the initial portions of the dextro- and levocardiograms do not develop at a constant velocity along straight lines, or that heating or cooling alters the velocity of development of the dextro- or levocardiogram.

Fig. 1a. Feb. 12, 1941. 10.5 kgm. Male dog. An enlarged photograph of a single complex taken from lead II after 12 minutes of heating the left ventricle at 50°C. This shows a reduction in the height of R and the plateau postulated in figure 1, no. 2.

METHOD. In the experiments reported in this paper, the hypothesis presented above concerning the formation of the R complex was tested by methods which presumably altered the interval separating the appearance of the dextro- and levocardiograms. Diminishing this interval should reduce the amplitude of R, while increasing the interval should augment R

by permitting the development of more of the upstroke of the dextrocardiogram before interference from the levocardiogram (fig. 1). Furthermore, if the levocardiogram should begin first, the initial deflection of the R complex should be downward. The amplitude of this complex should also depend upon the interval elapsing between the onset of the levocardio-

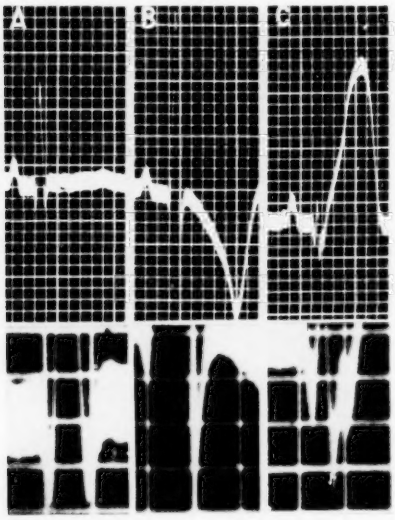


Fig. 2

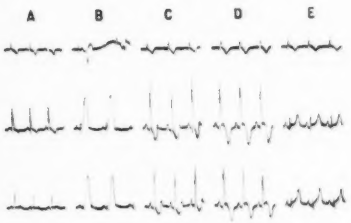


Fig. 3

Fig. 2. May 10, 1940. 7.5 kgm. dog. Enlarged photographs of electrocardiograms (lead III). (a) control, (b) after cooling the left ventricle, showing increased amplitude of R, and increase in the QR and QRS interval; (c) after heating the left ventricle, showing reduced amplitude of R, and shortening of the QR and Q-S interval. Below are greater enlargements of the Q-S interval.

Fig. 3. Nov. 24, 1940. 7.0 kgm. dog. Tracings of electrocardiograms which show the influence of heat and cold on the amplitude of the R complex. Tracings were employed because of the great amplitude of R in C and D and the consequent difficulty of adequate photographic reproduction. A. Control, in leads I, II, and III. B. After heating the left ventricle (50°C.). (Extrasystoles are seen in lead I.) C. Heating the right ventricle. D. Cooling the left ventricle (6°C.). E. Cooling the right ventricle.

gram and the onset of the dextrocardiogram. Two methods were employed. The first consisted in heating and cooling individual ventricles for several minutes in an endeavor to accelerate or delay the appearance of the excitatory process at the surface. The second method consisted in eliciting extrasystoles from symmetrically located points on the right and left ventricles equidistant from the septum. By this method the major portion of one or the other ventricle could be activated before the wave of

excitation reached the opposite ventricle. Bipolar electrodes, with an interpolar distance of 2 to 3 mm. were stitched to the epicardium and activated by a thyratron stimulator. Ten dogs were employed and were prepared as previously described (3).

RESULTS. A. *Effect on the R complex of heating and cooling individual ventricles.* Enlarged photographs of three typical R complexes are shown in figure 2; the first is the control complex (2A), the second (2B) one of increased amplitude produced by cooling the left ventricle, and the third (2C) a complex of low amplitude which followed warming the left ventricle. It can be seen that in the augmented R complex produced by cooling the left ventricle, the Q-R interval is increased, as might be expected if the onset of the levocardiogram were delayed. The Q-R (end of Q to apex of R) interval is decreased in the complex of low amplitude which was produced by heating the left ventricle. This is consistent with the supposition that the levocardiogram was initiated sooner. The influence of these alterations in Q-R upon the duration of the Q-S interval is shown in greater detail below the photographs of the whole complex.

The influence of thoroughly heating and cooling each ventricle on the amplitude of R is shown in figure 3. When the left ventricle was heated, R decreased in amplitude (3b) while its height increased when this ventricle was cooled (3d). Conversely, cooling the right ventricle diminished the height of R (3e) while warming increased it (3c). Consistent results were obtained in all experiments and could be reproduced as often as desired in each experiment. In some instances the increase above normal in the height of R was not striking, while the diminution was more readily obtained. The typical T wave changes produced by heat and cold consistently made their appearance as previously described (3, fig. 3). The changes in the R complex *a*, had a longer latency; *b*, required more time for their full development, and *c*, subsided more slowly after removal of the thermal chamber than the T wave changes which also appeared in response to heating or cooling. These differences in the evolution of changes in the R and the T complexes are summarized in figure 4.

B. *Direction of the R complex in extrasystoles from selected areas of right and left ventricles, equidistant from septum.* Figure 5 shows in confirmation of the theory presented in the introduction that extrasystoles elicited from the left ventricle exhibit a downward initial deflection in all three conventional leads (5b) while extrasystoles from the right ventricle show an initial deflection which is upward in all three leads (5a). One condition was essential to obtain such complexes, i.e., that the point of stimulation be sufficiently removed from the septum to permit spread of the impulse to the greater part of the ventricle stimulated before the opposite ventricle was involved. For this reason points were chosen approximately equidis-

tant from the septum. The configuration of extrasystoles elicited from points nearer to the septum is discussed in a following paper (4).

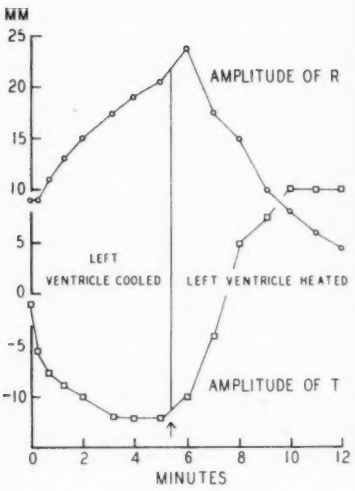


Fig. 4

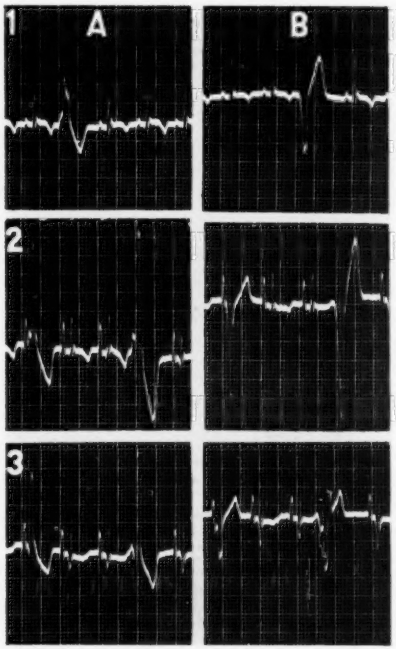


Fig. 5

Fig. 4. Same experiment as figure 1a showing the dissociation of R and T changes. In this figure are plotted the amplitude of R and T during cooling and then heating the left ventricle. Ordinates, amplitude of R and T in millimeters, abscissae, time in minutes. It is seen 1, that changes in the amplitude of T begin promptly when cooling starts while R remains for a time unchanged; 2, that the T wave reaches a maximum while the R wave continues to develop; 3, that the T wave responds promptly to the change from cooling to heating, while R continues to increase for a time before responding to cold; 4, that the T wave again levels off while R continues to change.

Fig. 5. Same experiment as figures 1a and 5. Electrocardiograms from the three leads, A showing extrasystoles elicited from the right ventricle, and B from the left ventricle. The points of stimulation were symmetrically placed on the right and left ventricles equidistant from the septum. The right ventricular extrasystole is initiated by an upright deflection in all three leads, while the left ventricular extrasystole begins with a downward initial deflection in all three leads.

SUMMARY. 1. Heating the left ventricle or cooling the right ventricle for a sufficient time, shortened the Q-R interval and decreased the amplitude of R.

2. Heating the right ventricle or cooling the left ventricle lengthened the Q-R interval and increased the amplitude of R.

3. Extrasystoles elicited by primary activation of the major part of the left ventricle showed downward initial deflections in all three conventional leads.

4. Extrasystoles elicited by primary activation of the major part of the right ventricle exhibited an upright initial deflection in all three conventional leads.

CONCLUSIONS

1. The R complex of the electrocardiogram results from the algebraic summation of the initial portions of the dextro- and levocardiograms.

2. When the R complex is upright, its initial deflection is the upstroke of the dextrocardiogram, while the downstroke is produced by the onset and development of the levocardiogram.

3. When the R complex is directed downward, the downstroke is the initial portion of the levocardiogram, while the upstroke is produced by the onset and development of the dextrocardiogram.

4. The amplitude of the R complex varies with the interval separating onset of the dextro- and levocardiograms. The maximum amplitude is limited by the amplitude of the component dextro- or levocardiograms.

REFERENCES

(1) Hoff, H. E., L. H. Nahum and B. Kisch. This Journal **131:** 687, 1941.
(2) Wiggers, H. C. This Journal **118:** 333, 1937.
(3) Hoff, H. E. and L. H. Nahum. This Journal **131:** 700, 1941.
(4) Nahum, L. H., H. E. Hoff and W. Kaufman. This Journal **134:** 398, 1941.

THE NATURE OF LEADS I AND III OF THE ELECTROCARDIOGRAM[1]

H. E. HOFF, L. H. NAHUM AND W. KAUFMAN[2]

From the Laboratory of Physiology, Yale University School of Medicine, New Haven, Conn.

Accepted for publication, June 25, 1941

Findings reported in a foregoing paper permit the deduction that different areas of the heart are represented in leads I and III of the electrocardiogram (1). The reasoning upon which this deduction is based is as follows:

When the electrical activity of the anterior surface of the heart is abolished by a potassium pledget covering portions of both ventricles, a dextrocardiogram is recorded in lead I and a levocardiogram in lead III. Lead II may show a practically normal complex. With such treatment of the anterior surfaces, only the posterior surfaces of the ventricles can be the source of action potentials; therefore, the dextrocardiogram in lead I must be derived from the posterior surface of the right ventricle (posterior dextrocardiogram). The presence of a levocardiogram in lead III indicates similarly that lead III has recorded from the posterior surface of the left ventricle (posterior levocardiogram). When the posterior surfaces of the ventricles are treated with potassium to abolish action potentials from this region, a levocardiogram is obtained in lead I, while a dextrocardiogram is found in lead III. Lead II again may show only minor deviations from the control, provided that equal surfaces of the right and left ventricles are treated. In this case the levocardiogram in lead I must be recorded from the anterior surface of the left ventricle (anterior levocardiogram), while the dextrocardiogram in lead III must be derived from the anterior surface of the right ventricle (anterior dextrocardiogram).

The inference may therefore be drawn that 1, contiguous regions of the right and left ventricles do not participate in the interference which produces the electrocardiogram in leads I and III; 2, lead I records, at least preponderantly, the interference between the action potentials of the anterior surface of the left ventricle, and the posterior surface of the right ventricle (i.e., lead I = posterior dextrocardiogram + anterior levocardio-

[1] Aided by grants from Fluid Research Funds, Yale University and Emanuel Libman Fellowship Fund.

[2] Fellow of the Dazian Foundation.

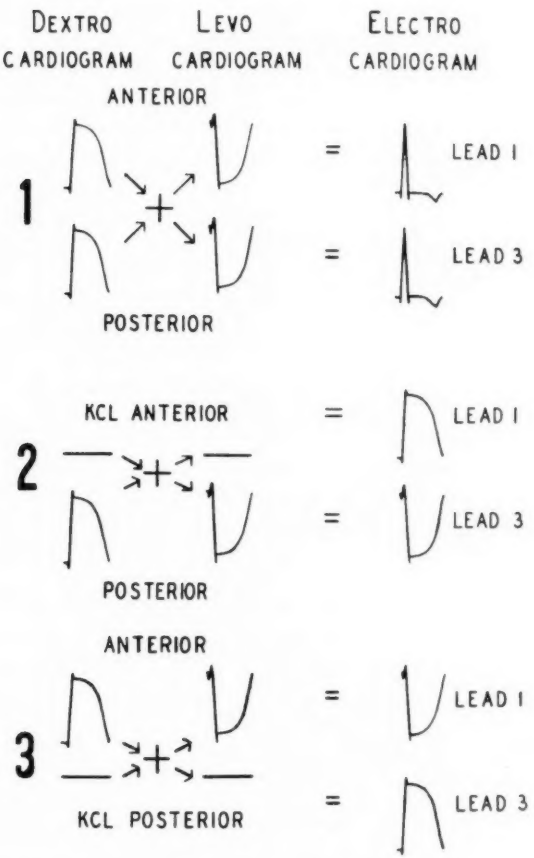


Fig. 1. A diagram illustrating in section 1 the hypothesis that lead I is formed by the algebraic summation of the anterior levocardiogram and the posterior dextrocardiogram, while lead III is formed by the summation of the anterior dextrocardiogram and the posterior levocardiogram. In this nomenclature it is assumed that each ventricle can be divided into an anterior and posterior region. Dextro- and levocardiograms are drawn as they are most frequently recorded. A Q wave is seen in the dextrocardiogram and a Q and small R are seen in the levocardiogram. These waves are included because they indicate the slightly earlier onset of the main initial deflection of the dextrocardiogram compared with the levocardiogram. They are not to be interpreted as an integral component of either the dextro- or levocardiogram. The diagram in section 2 explains the effect of abolishing action potentials from the anterior surfaces of both ventricles by the application of KCl. The diagram in section 3 explains the effect of abolishing the posterior dextro- and levocardiograms by KCl.

gram); 3, lead III records the interference between the action potentials of the anterior surface of the right ventricle and the posterior surface of the left ventricle (lead III = anterior dextrocardiogram + posterior levocardiogram); 4, lead II appears to record from the entire heart, being the summation of effects in leads I and III. Figure 1 illustrates these conclusions. In the following experiments further evidence is developed in support of the theory that leads I and III record from different surface areas of the heart as described above in 2 and 3.

A. *Leads in which T wave changes appear when specific areas of the surface of the heart are heated and cooled.* Heat and cold are known to shorten and lengthen, respectively, the dextro- or levocardiogram, thereby producing characteristic T wave changes (2). When heat or cold is applied to the surface of the heart the characteristic T wave changes which result serve to identify the ventricle so treated. In the following experiments, contiguous areas of both the right and left ventricles were treated simultaneously, to determine the nature of the T wave changes in leads I and III. In other experiments portions of the surface of a single ventricle were heated and cooled to determine the lead in which the resulting T wave changes appeared. A third procedure consisted in producing T wave changes in a single lead by thermal application to a chosen area of one ventricle and then exploring the opposite ventricle with a second thermal chamber to find the region which interferes with the effects of the first application. Ten dogs were employed, prepared as described previously (2).

Results of heating and cooling selected areas of the surface of the heart. When the anterior surface of the heart was cooled by a thermal chamber covering portions of both right and left ventricles, oppositely directed T waves appeared as follows: *a*, a prolonged, inverted T wave appeared in lead I, indicating in this lead the influence of a prolonged levocardiogram which could have been derived only from the cooled anterior surface of the left ventricle; *b*, in lead III the T wave was upright and prolonged, indicating the influence in this lead of a prolonged dextrocardiogram which could have been derived only from the anterior surface of the right ventricle (fig. 2 C). Lead I therefore must have recorded preponderantly from the anterior surface of the left ventricle, while lead III must have recorded from the anterior surface of the right ventricle (fig. 3, section 1). The results obtained by warming the anterior surface also showed oppositely directed T waves in leads I and III (fig. 2 B). In this case, however, T_I was upright, indicating shortening of the anterior levocardiogram, while T_{III} was inverted, indicating curtailment of the anterior dextrocardiogram. This substantiates the hypothesis that the anterior levocardiogram is recorded in lead I, while the anterior dextrocardiogram is recorded in lead III (fig. 3, section 3).

Warming the posterior surfaces of both ventricles produced an inverted

T wave in lead I and an upright T wave in lead III (fig. 2 E). The inverted T wave in lead I indicates the existence of a shortened dextrocardiogram which could have been derived only from the warmed posterior surface of the right ventricle. The upright T wave in lead III indicates a shortening of the posterior levocardiogram, which could have been derived only from the warmed posterior surface of the left ventricle (fig. 3, section 4).

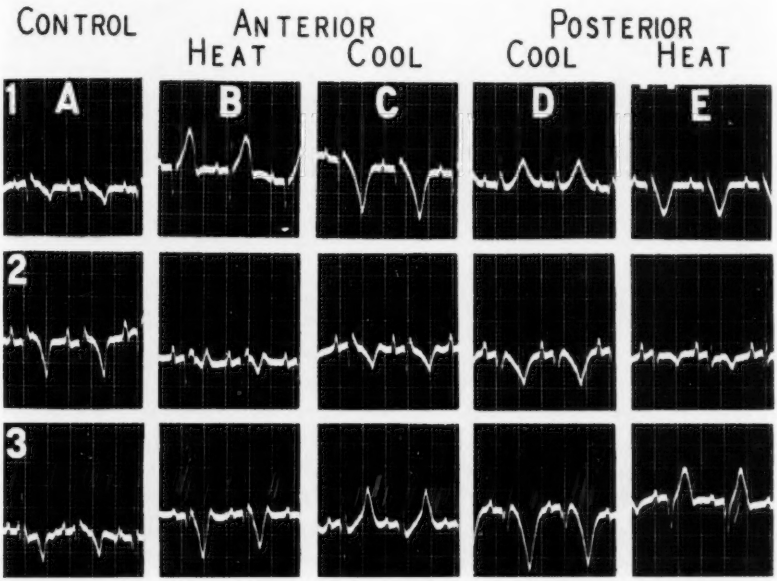


Fig. 2. Dog. Feb. 7, 1941. A. Control, leads I, II, and III. B. The influence of heating the anterior surfaces of the right and left ventricles (50°C.). T_I is sharply upright and T_{III} sharply inverted. Q-T duration unchanged. C. Cooling (5°C.) the anterior surfaces of both ventricles. T_I sharply inverted, T_{III} sharply upright. Q-T interval prolonged. D and E, comparable records obtained after cooling and heating the posterior surfaces of both ventricles.

Cooling the posterior surfaces of both ventricles produced an upright T_I and an inverted T_{III} (fig. 2 D). The upright T_I indicates the presence of a prolonged dextrocardiogram, which could have been derived only from the cooled posterior surface of the right ventricle. The inverted T_{III} similarly indicates the presence of a prolonged levocardiogram, which must have been derived from the cooled posterior left ventricle (fig. 3, section 2).

These experiments, which are summarized in figure 3, indicate that lead I records predominantly from the anterior surface of the left ventricle, and the posterior surface of the right ventricle (anterior levocardiogram

and posterior dextrocardiogram) whereas in lead III is recorded preponderantly the electrical activity of the anterior surface of the right ventricle, and the posterior surface of the left ventricle (anterior dextrocardiogram and posterior levocardiogram).

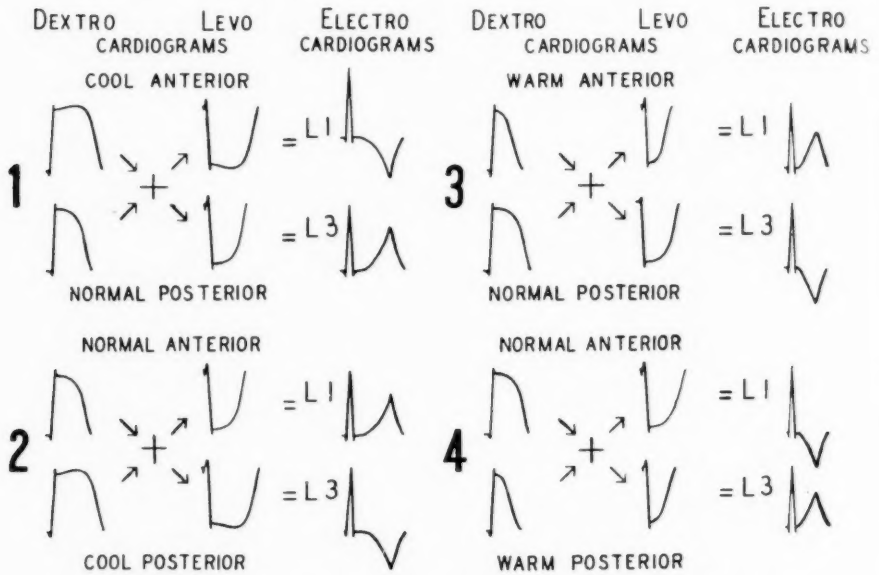


Fig. 3. A diagram illustrating the mechanism of changes produced in the electrocardiogram by heating and cooling the surface of the heart, to be compared with actual records in figure 3. In section 1 the anterior surface is cooled, prolonging the anterior dextro- and levocardiograms. Summation as proposed in the theory developed in this paper produces an inverted T_I and an upright T_{III}. The Q-T interval is prolonged in both leads. Warming the same region (section 3) shortens the anterior dextro- and levocardiograms, and results in an upright T_I and an inverted T_{III}. The duration of Q-T is unchanged in both leads. In sections 2 and 4 the influence of cooling and warming the posterior surfaces of both ventricles is illustrated. For the purpose of simplification, the time of onset of the dextro- and levocardiograms is not changed in the diagrams. In actual experiments, heating or cooling does eventually alter the time of onset of the dextro- and levocardiograms, and produces changes in the amplitude of the R complex (see figs. 4 and 5).

A further test of the validity of the hypothesis presented here consisted in producing characteristic alterations in the T wave by warming or cooling the anterior or posterior surface of a single ventricle to determine *a*, in which lead the effect was manifest, and *b*, where on the other ventricle a similar simultaneous thermal application would neutralize the first alterations. It was found that lead I recorded changes due to heating and

cooling the anterior left and posterior right ventricular surfaces while lead III reflected events at the anterior right and posterior left ventricular surfaces.

The effects of applications of heat or cold to the anterior surface of the right ventricle, which appear predominantly in lead III, were neutralized by a similar application to the posterior surface of the left ventricle. Likewise, heating or cooling the anterior surface of the left ventricle interfered with the effects of similar thermal changes at the posterior surface of the right ventricle. These changes appeared predominantly in lead I, but completely satisfactory records of this interference were not obtained, due to difficulty in placing and maintaining the thermal chamber in a proper position on the posterior surface of the right ventricle.

B. *Alterations in the R complex of leads I and III produced by heating and cooling anterior and posterior surfaces of both ventricles.* The R complex results from the algebraic summation of the initial portions of the dextro- and levocardiograms (3). The upstroke to the peak of R is formed by the action current of the right ventricle, while the downstroke is developed by the onset of activity in the left ventricle. The height of R is therefore in part determined by the interval between the activation of the two ventricles (3). If this interval is shortened, the amplitude of R must decrease, while if it is lengthened, the amplitude should increase (3). Application of cold or heat to a ventricle will, after a sufficient interval, alter the arrival of the cardiac impulse at the surface of the region treated, delaying or hastening its activation in relation to the other ventricle, and thus produce characteristic alterations in the amplitude of R (3). When, then, areas of both ventricles are simultaneously heated or cooled, the resulting changes in R will indicate which ventricle is responsible for them.

Method. Heating and cooling of the anterior and posterior surface of the heart involving both ventricles was carried out in 7 experiments as described previously. The resulting changes in the height of R were determined.

Results. Figures 4 and 5 illustrate the influence on the height of the R complex in leads I and III of heating and cooling anterior and posterior septal regions. Heating the anterior surface reduced the amplitude of R_I and increased R_{III} (fig. 4 C) while cooling had the opposite effect (fig. 4 B). Conversely, heating the posterior surface increased the amplitude of R_I and reduced R_{III} (fig. 5 C); cooling decreased R_I and increased R_{III} (fig. 5 B). The significance of these results is discussed in the following paragraphs.

When the posterior surfaces of both ventricles were cooled the amplitude of R_I diminished while the amplitude of R_{III} increased. Cooling necessarily delayed the arrival of the impulse to the two posterior surfaces and produced a delayed posterior dextrocardiogram and a delayed posterior

levocardiogram. The influence of the delayed posterior dextrocardiogram was found in lead I in the reduced amplitude of R. This indicates that the posterior dextrocardiogram is recorded selectively in lead I. The influence of the delayed posterior levocardiogram was found in the increased amplitude of R in the lead III. The posterior levocardiogram is therefore shown to appear selectively in lead III.

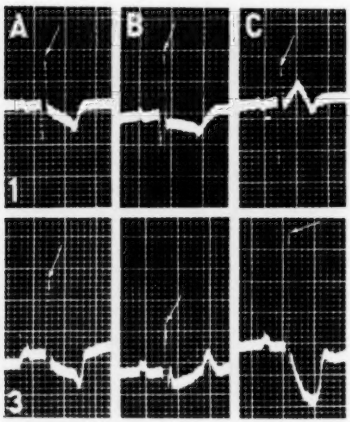


Fig. 4

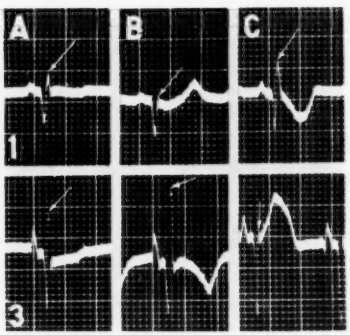


Fig. 5

Fig. 4. Dog. 10 kgm. April 25, 1941. Leads I and III. Arrows point to apex of R complex. Unretouched photographic enlargements.

A. Control with thermal chambers at body temperature in place on anterior surfaces of right and left ventricle.

B. Cooling (0°C.). Increased R_I, diminished R_{III}.

C. Warming (48°C.). Marked reduction of R_I, increase in R_{III}.

Fig. 5. Dog. 11.3 kgm. April 23, 1941. Leads I and III. Arrows point to apex of R complex. Unretouched photographic enlargements.

A. Control. Thermal chambers at body temperature in place on posterior surfaces of the right and left ventricles.

B. Cooling (0°C.). Reduction in R_I and increase in R_{III}.

C. Heating (48°C.). R_I increased, R_{III} diminished.

Heating the posterior surfaces of both ventricles produced an augmented R_I and diminished R_{III}. Warming these regions hastened the initiation of the posterior dextro- and levocardiograms. The influence of the premature onset of the posterior dextrocardiogram was shown in the increased amplitude of R_I, indicating that the posterior dextrocardiogram is recorded selectively in lead I. The influence of the premature onset of the posterior levocardiogram was shown in the decreased amplitude of R_{III}, indicating that the posterior levocardiogram is recorded selectively in lead III.

Similar reasoning applied to the influence of heating and cooling the anterior surface of both ventricles on the amplitude of R_I and R_{III} permits the deduction that the anterior dextrocardiogram is recorded selectively in lead III and the anterior levocardiogram is recorded selectively in lead I.

In these experiments, as in those reported in the previous section, lead II recorded the approximate algebraic summation of lead I and lead III.

SUMMARY. 1. Cooling the anterior surfaces of both right and left ventricles produced an inverted prolonged T_I and a prolonged upright T_{III}. R_I was increased and R_{III} was diminished in amplitude.

2. Cooling the posterior surfaces of both right and left ventricles produced a prolonged upright T_I and a prolonged inverted T_{III}. R_I was diminished and R_{III} augmented.

3. Heating the anterior surfaces of both right and left ventricles produced an upright T_I of normal duration and an inverted T_{III} of normal duration. R_I was decreased in amplitude and R_{III} was increased.

4. Warming the posterior surfaces of both right and left ventricles produced an inverted T_I of normal duration and an upright T_{III} of normal duration. R_I was increased and R_{III} decreased in amplitude.

CONCLUSIONS

1. Lead I records the algebraic summation of the anterior levocardiogram and the posterior dextrocardiogram.

2. Lead III records the algebraic summation of the anterior dextrocardiogram and the posterior levocardiogram.

REFERENCES

(1) NAHUM, L. H., H. E. HOFF AND B. KISCH. This Journal **131:** 687, 1941.
(2) HOFF, H. E. AND L. H. NAHUM. This Journal **131:** 693, 1941.
(3) NAHUM, L. H., H. E. HOFF AND W. KAUFMAN. This Journal **134:** 384, 1941.

CONFIGURATION OF ANTERIOR AND POSTERIOR SEPTAL EXTRASYSTOLES IN THE STANDARD LEADS OF THE ELECTROCARDIOGRAM[1]

L. H. NAHUM, H. E. HOFF AND W. KAUFMAN[2]

From the Laboratory of Physiology, Yale University School of Medicine, New Haven, Conn.

Accepted for publication June 25, 1941

In the experiments to be reported here, a study was made of the configuration of extrasystoles elicited from the septum and immediately adjacent regions. It would be expected that the radial spread of an impulse starting at or near the septum would produce simultaneous excitation of adjacent areas of both right and left ventricles. Thus, an impulse originating at the anterior septum midway between apex and base would be expected to excite the anterior portions of the right and left ventricle before it reached the posterior surfaces of the heart. Conversely, a stimulus applied to the posterior septum would be expected to evoke simultaneous response in the posterior surfaces of the right and left ventricle before the anterior surfaces were activated.

Evidence has already been presented (1) that lead I records the interference between the anterior levocardiogram and the posterior dextrocardiogram. Lead III records a similar interference between the posterior levocardiogram and the anterior dextrocardiogram. According to this formula, extrasystoles originating in the anterior or posterior septal regions should exhibit predictable configurations. When an extrasystole is elicited by stimulation of the anterior septum, thus exciting simultaneously the anterior surfaces of the right and left ventricles, the anterior levo- and dextrocardiograms should both begin before their posterior counterparts. Therefore, the first electrical activity of these extrasystoles recorded in lead I should be the unopposed downstroke of the anterior levocardiogram. Similarly, the first electrical activity of the extrasystole appearing in lead III should be the unopposed upstroke of the anterior dextrocardiogram (fig. 1A).

By similar reasoning it may be predicted that posterior septal extrasystoles should show an upward initial deflection in lead I derived from

[1] Aided by grants from Fluid Research Funds, Yale University and Emanuel Libman Fellowship Fund.

[2] Fellow of the Dazian Foundation.

the unopposed upstroke of the posterior dextrocardiogram and a downward deflection in lead III derived from the unopposed initial downward deflection of the posterior levocardiogram (fig. 1B).

METHOD. Six dogs were employed. They were prepared as described previously (1). Extrasystoles were elicited by delivering periodic break

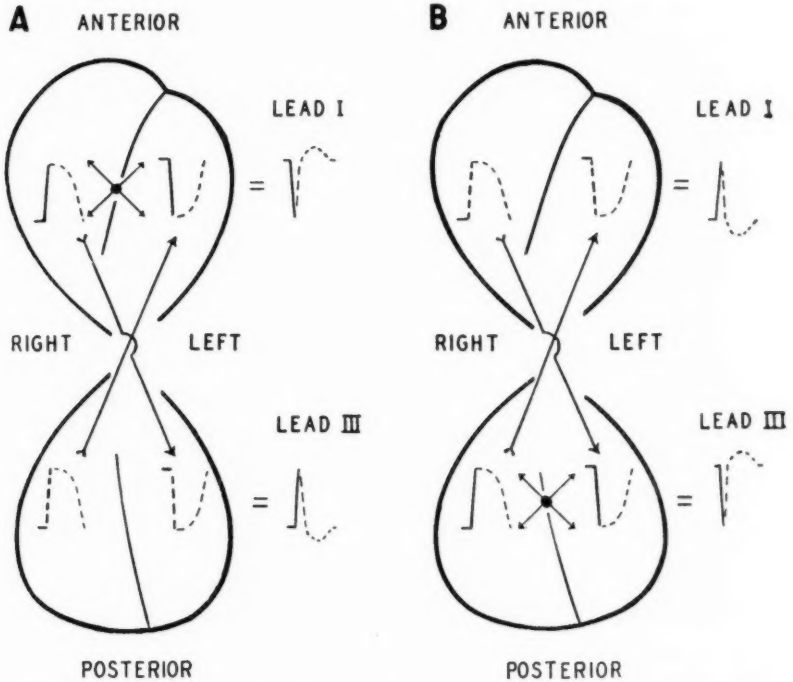


Fig. 1. A diagram illustrating the configuration of anterior and posterior septal extrasystoles. An extrasystole originating at the anterior septum (A) will involve the anterior surfaces of both right and left ventricles before spreading to the posterior surfaces, as is indicated by the heavy lines of the anterior dextro- and levocardiograms. Since the anterior levocardiogram is recorded in lead I, the initial deflection in this lead will be downward. The anterior dextrocardiogram is recorded in lead III, producing an initial upward deflection in this lead. A similar explanation for the configuration of posterior septal extrasystoles is presented in B.

shocks from a thyratron stimulator through bipolar electrodes stitched to the epicardium.

RESULTS. Figure 2 shows the configuration of extrasystoles in the three leads when the anterior surfaces of both ventricles were activated first (C), and when the initial activation involved the posterior surfaces of

both ventricles (D). As predicted above, the initial deflection of the ectopic beats from the anterior septum was downward in lead I and upward in lead III. The initial deflection of extrasystoles evoked by stimulation of the posterior septum was directed upward in lead I and downward in lead III. Consistent with other evidence (3) is the finding that extra-

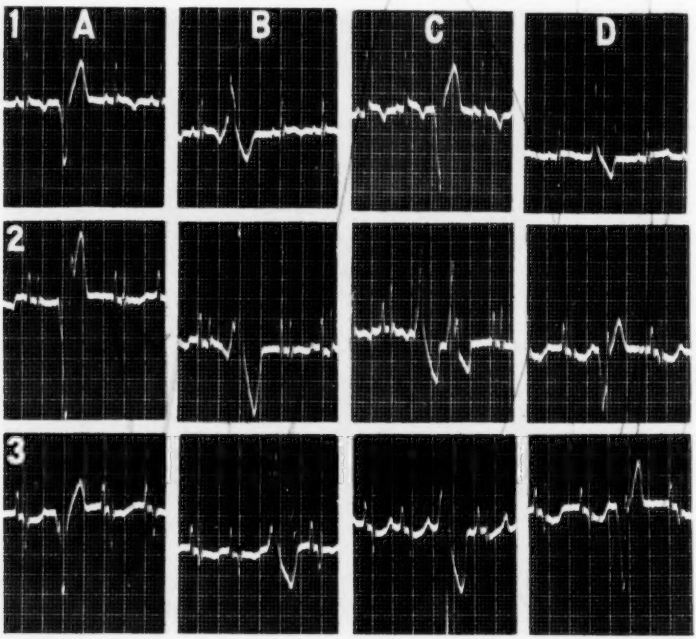


Fig. 2. Dog. Feb. 12, 1941. A, leads 1, 2, 3, showing extrasystoles elicited by bipolar stimulation near the center of the surface of the left ventricle. Initial deflections downward in all three leads. B, extrasystoles from the center of the right ventricular surface, showing an upright initial deflection in all three leads. C, extrasystoles elicited from the mid-point of the anterior septum. A downward initial deflection in lead I and an upright initial deflection in lead III. D, extrasystoles elicited from the posterior septum. Upright initial deflection in lead I and downward initial deflection in lead III.

systoles from the septum at the apex have the same configuration as extrasystoles from the posterior septum.

Extrasystoles elicited from regions lying between the lateral areas, which gave complexes with the same direction in all three leads, and the septal areas giving the oppositely directed complexes in leads I and III as described above, showed transitional patterns which will be discussed in another communication.

Discussion. The configuration of extrasystoles obtained in these experiments is consistent with the theory that distinct regions of the heart are selectively represented in leads I and III; i.e., lead I records the algebraic summation of the anterior levocardiogram and the posterior dextrocardiogram, while lead III records a similar summation of the anterior dextrocardiogram and the posterior levocardiogram.

These experiments, together with those reported previously (2), permit certain statements concerning the origin of ventricular extrasystoles. A downward initial deflection in lead I indicates that the anterior surface of the left ventricle was excited before the posterior surface of the right ventricle, while an upward initial deflection in this lead indicates that the posterior surface of the right ventricle was excited before the anterior surface of the left ventricle. A downward initial deflection in lead III indicates that the posterior surface of the left ventricle was excited before the anterior surface of the right ventricle, while an upward initial deflection in this lead indicates that the anterior surface of the right ventricle was excited before the posterior surface of the left ventricle.

An upward initial deflection in both leads I and III indicates that the major portion of the right ventricle was excited in advance of the left ventricle. Conversely, a downward initial deflection in both leads I and III indicates that the major portion of the left ventricle was excited before the right ventricle. Such configurations arise when extrasystoles originated near the center of the ventricular surface (2).

When the initial deflections of an extrasystole are oppositely directed in leads I and III, either the anterior or posterior surfaces of both ventricles must have been excited more or less simultaneously, and in advance of the opposite surfaces. Extrasystoles originating at or near the anterior septum exhibit a downward initial deflection in lead I and an upward initial deflection in lead III. Extrasystoles originating at or near the posterior septum show an upward initial deflection in lead I and a downward initial deflection in lead III.

Electrocardiograms showing in lead I an upward QRS and in lead III a downward initial deflection are now said to indicate "left ventricular preponderance," or "left axis deviation." A downward initial deflection in lead I and an upward initial deflection in lead III are termed "right ventricular preponderance," or "right axis deviation." It can be seen from the experiments reported above that when the anterior surfaces of both ventricles are activated before the posterior surfaces, the direction of the initial deflection in leads I and III is what would be interpreted as "right axis deviation." Conversely, primary activation of the posterior surfaces of both ventricles gives rise to configurations which are interpreted as "left axis deviation."

It is also interesting to compare the patterns of extrasystoles from the

anterior or posterior septum with electrocardiograms interpreted as indicating right or left bundle-branch block. The pattern of upward initial deflection in lead I and a downward initial deflection in lead III, characteristic of left bundle-branch block, is also characteristic of extrasystoles produced by primary activation at the posterior surface, while a downward initial deflection in lead I and an upward deflection in lead III, characteristic of right bundle-branch block, would in an extrasystole signify the primary activation of the anterior surfaces of both ventricles.

SUMMARY

1. Extrasystoles elicited from the anterior septum show a downward initial deflection in lead I and an upward initial deflection in lead III.

2. Extrasystoles elicited from the posterior septum and apex show an upward initial deflection in lead I and a downward initial deflection in lead III.

3. These findings support the theory that lead I records the algebraic summation of the anterior levocardiogram and the posterior dextrocardiogram, while lead III records the summation of the anterior dextrocardiogram and the posterior levocardiogram.

4. Extrasystoles from the anterior septum are comparable with ventricular complexes at present interpreted as having "right axis deviation" or "right bundle-branch block."

5. Extrasystoles from the posterior septum are comparable with ventricular complexes interpreted as showing "left axis deviation" or "left bundle-branch block."

REFERENCES

(1) Hoff, H. E., L. H. Nahum and W. Kaufman. This Journal **134:** 384, 1941.
(2) Nahum, L. H., H. E. Hoff and W. Kaufman. This Journal **134:** 390, 1941.
(3) Nahum, L. H., H. E. Hoff and B. Kisch. This Journal **131:** 693, 1941.

THE VERTICAL BALLISTOCARDIOGRAPH; EXPERIMENTS ON THE CHANGES IN THE CIRCULATION ON ARISING; WITH A FURTHER STUDY OF BALLISTIC THEORY

ISAAC STARR AND A. J. RAWSON

From the Research Department of Therapeutics and the Johnson Foundation of the University of Pennsylvania

Accepted for publication June 30, 1941

Progress in the physiological aspects of our work with the ballistocardiograph (Starr, Rawson, Schroeder and Joseph, 1939) has taken three directions. Theoretical studies on the origin of the impacts have been continued, leading to a change in certain of our views and the clarification of others.

A second ballistocardiograph has been constructed to obtain records in the standing or sitting position.

By this means we have studied the changes occurring in the cardiac output immediately after assuming the erect position, a time when some normal persons and many patients exhibit transient symptoms of dizziness or lightheadedness. Comparisons of the cardiac output lying and standing have been made by many students of the subject, but the results obtained have differed. This literature has been reviewed by McDowell (1938).

We have made over 100 estimations on 58 normal subjects. Our results show that the average cardiac output per minute remains the same after assuming the erect posture, although some individuals consistently have smaller cardiac outputs per minute standing than lying. When the circulation in reclining subjects is above average normal it tends to diminish when the subject stands; when below average normal, it tends to remain the same or to rise. This same tendency can be demonstrated in many of the published results and it will explain some of the discrepancies found.

We have also studied methods of changing the relationship between the amount of the circulation in the two positions. This could be greatly altered by the drug paredrine, and also by the application of an abdominal binder in certain subjects.

Our theoretical studies were much assisted by Dr. LeRoy Williams who made two casts of the ventricular chambers for us and permitted us to make measurements in the dissecting room. We are also indebted to Dr. Hugo Roesler for advice concerning the position of the axes of the cardiac chambers.

STUDIES ON BALLISTIC THEORY. *A mechanical analogy.* Most readers will recall what happens when a long freight train, stopped on the track, is started by the locomotive. The train is not set in motion as a unit, but car by car, the impulse traveling down the train so that the last car moves some time after the first has started. Bend such a train around a curve like the aortic arch, have the locomotive push rather than pull, have it start, move a short distance and stop again, assume a second train for the pulmonary system and one has a reasonably close analogy to what happens in the circulation. The impacts of such a system will be the sum of the impacts of its units (cars) and these will be delivered in various directions due to the curve of the track and at different times as each starts and stops a little later in time than the one before it.

We propose to analyze the ballistics of the circulation according to this analogy by dividing it into units, analogous to the cars, the impulse traveling down the train as it starts being analogous to the pulse wave velocity. Thus we will estimate the impacts which would arise from the moving blood of subject Sta. during a single systole. This subject was chosen, not only for convenience, but also because he had proved to be an average subject in our previous investigation (Starr et al., 1939).

Construction of a schematic aorta. A diagram of the aorta of Sta. (fig. 1, A) was constructed from expectations based on Bazett's data, the relative measurements being influenced by those obtained from a cadaver of similar age found in the dissecting room. Great exactitude was not believed possible.

The section area of the schema's ascending aorta before the branches was placed at 3.64 sq. cm., the size expected from Bazett's data. The volume of the ascending aorta and the arch is 64 cc. while the nearest corresponding figure in Bazett's data, which includes the volume of the branches for some distance, is 81 cc. The volume of the schema's thoracic and abdominal aorta to the bifurcation is 80 cc. which can be compared with 101 cc. in Bazett's data, the latter figure including the femorals and the mouths of the larger branches.

In figure 1, A we have sought to divide this schematic aorta into cylindrical segments the contents of which would move to the segment next beyond whenever 10 cc. left the heart. Having no branches, the ascending aorta has been divided into segments of 10 cc. each. When the branches are reached, a part of the blood leaves the aorta and we have assumed that the blood thus diverted to the segmental vessels was proportional to the diminution in size of the aorta found in the cadaver, and continued this conception to the bifurcation. The volumes of segments used in the calculation but not shown in figure 1, A, from A XI to A XX, are: 5.6, 5.3, 5.0, 4.8, 4.4, 4.1, 3.9, 3.6, 3.3, and 3.1 cc.

A schema of the pulmonary artery divided into similar segments is also given in figure 1, B.

Relations of space and time. Thus in figure 1, A one can visualize the positions which the various units of blood will occupy *in space* as the heart contracts. Equally important is the *time* at which the blood units reach the successive positions. To obtain the latter, the area under Machella's (1936) blood velocity curve (fig. 1, C) was estimated by drawing it on cross section paper and counting the squares, and this area was divided by vertical lines into 6 equal parts (fig. 1, C). The intersections of these dividing lines with the abscissa are labeled Instant 1, 2, etc., and they indicate the times at which a unit of blood reaches the successive positions. Thus the blood occupying the proximal position in the aorta (A I, fig. 1, A) before systole begins at Instant I in time, will occupy, in succession, position A II in space at Instant 2 in time, A III at Instant 3, etc.

Calculation of impacts from blood in the great vessels. To calculate the impact of any blood unit in the aorta, we need first to determine its longitudinal velocity, next its longitudinal acceleration, and then to multiply the ordinates of the acceleration curve by the mass concerned. The method used for all the units can be illustrated by calculating the impacts arising from the blood in the proximal aorta, Unit A I, in its course around the aortic arch during a single systole.

In traveling from A I to A III in space and from Instant 1 to 3 in time, the course of Unit A I is longitudinal so Machella's (1936) curve is followed to Instant 3 in time. When traveling around the aortic arch, the longitudinal velocity diverges markedly from the true velocity and the former has been calculated by multiplying the ordinates of the true velocity curve by the cosines of the successive angles of divergence from the longitudinal. Angle Θ (fig. 1, A) which corresponds to this angle of divergence can be used more conveniently. The result is shown by the broken line in figure 1, C.

The next step is to calculate the longitudinal acceleration by differentiating the longitudinal velocity curve; the curve resulting is shown in figure 1, D.

We may now calculate the force of the impacts by multiplying the differential curve by the mass involved. The specific gravity of the blood being approximately 1, this remains 10 grams (1 mass unit) until the blood passes from position A III to A IV (fig. 1, A) during which change part of the blood is lost to the large branches, the mass remaining in the aorta diminishing as a result. Accordingly, to estimate the force of the impact we have multiplied the curve by a mass factor of 1 until the position A IV is attained where it is multiplied by 0.91. At A V, it is multiplied by 0.71, at A VI and VII by 0.67. Therefore the impact curve diverges from the acceleration curve as is shown by the dotted line in figure 1, D.

This method was employed for each of the segments in the aorta down to its bifurcation and for the pulmonary artery as well. Typical representatives of the family of impact curves resulting are shown in figure 1, F.

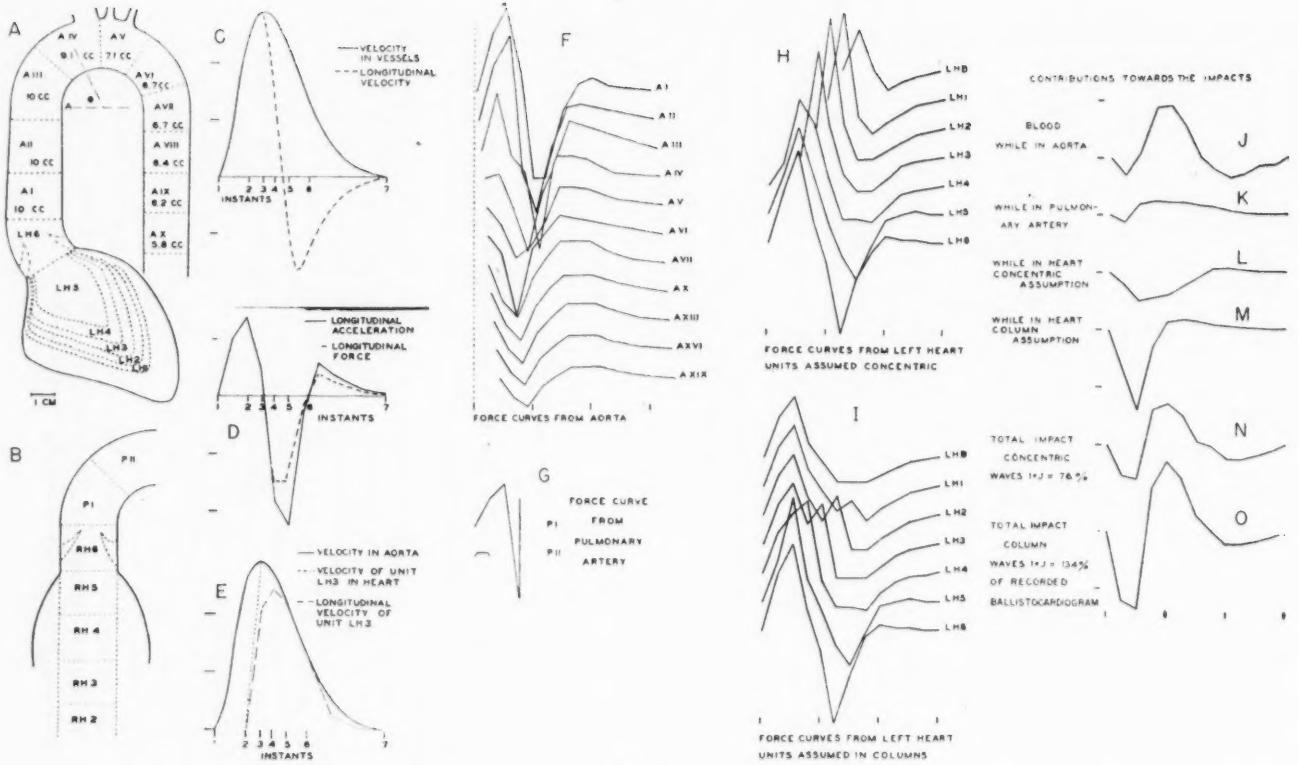

A
AIV
AV
AIII
AVI
10 CC
AVII
6.7 CC
AII
AVIII
10 CC
6.4 CC
AI
AIX
10 CC
6.2 CC
LH6
AX
5.8 CC
LH5
LH4
LH3
LH2
LH1
1 CM
B
PII
PI
RH6
RH5
RH4
RH3
RH2
C
VELOCITY IN VESSELS
LONGITUDINAL VELOCITY
INSTANTS
D
LONGITUDINAL ACCELERATION
LONGITUDINAL FORCE
INSTANTS
E
VELOCITY IN AORTA
VELOCITY OF UNIT LH3 IN HEART
LONGITUDINAL VELOCITY OF UNIT LH3
INSTANTS
F
AI
AII
AIII
AIV
AV
AVI
AVII
AX
AXIII
AXVI
AXIX
FORCE CURVES FROM AORTA
G
FORCE CURVE FROM PULMONARY ARTERY
PI
PII
H
LH8
LH1
LH2
LH3
LH4
LH5
LH6
FORCE CURVES FROM LEFT HEART
UNITS ASSUMED CONCENTRIC
I
LH8
LH1
LH2
LH3
LH4
LH5
LH6
FORCE CURVES FROM LEFT HEART
UNITS ASSUMED IN COLUMNS
CONTRIBUTIONS TOWARDS THE IMPACTS
BLOOD WHILE IN AORTA
J
WHILE IN PULMONARY ARTERY
K
WHILE IN HEART CONCENTRIC ASSUMPTION
L
WHILE IN HEART COLUMN ASSUMPTION
M
TOTAL IMPACT CONCENTRIC WAVES I+J = 78%
N
TOTAL IMPACT COLUMN WAVES I+J = 134% OF RECORDED BALLISTOCARDIOGRAM
O

Fig. 1

The final problem is to place these curves with relation to time. The units of blood in the aorta move in a sequence determined by the pulse wave velocity. To estimate this for any unit we have measured its distance from the aortic or pulmonary valves and then used average figures for pulse wave velocity given by Bazett (1940) for subjects of middle age, i.e., 3.3 meters per second, the heart to subclavian pulse wave velocity, for the ascending aorta, the transverse aorta, and the pulmonary artery; 7.6 meters per second, the subclavian to femoral velocity, for the descending aorta.

Calculation of impacts from blood in the heart. The position of the main axes, the lines from the centers of mass of the ventricular blood to the center of the outlet valve rings is clearly shown by Roesler's (1940) illustrations comparing x-ray pictures of hearts taken during life with dissections of the same organs post mortem. In most of the hearts there recorded the main axis of the left ventricle lies about 45° from the longitudinal axis of the body. The axis of the right ventricle deviates about 20° from the body's axis on the opposite side. Inspection of a large number of orthodiagrams with the same problem in mind gave the impression that the position of the left ventricular axis was usually at 45° but might often vary from 30° to 55°; the right from 35° to 5°. After inspection of his orthodiagram we accepted the values of 45° and 20° for the deviations of the axes of the cardiac chambers of subject Sta.

The maximum possible impacts of the blood in the heart would occur if all the units to be ejected formed a column whose end presented at the

Fig. 1. A. Schema of the aorta of subject Sta. Cardiac blood units grouped according to the "concentric" hypothesis.

B. Schema of the pulmonary artery of Sta. Cardiac units grouped according to the "column" hypothesis.

C. Solid line—Machella's (1936) blood velocity curve drawn to linear coördinates. Broken line—longitudinal velocity of blood Unit A 1 during a single systole. Derivation of instants of time in text.

D. Longitudinal acceleration and force curves for Unit A 1.

E. Derivation of longitudinal velocity of blood Unit LH 3 during its course from heart into aorta during a single systole.

F. Representative force curves from blood units in the aorta aligned according to the pulse wave velocity.

G. Similar force curves from the pulmonary artery.

H. Force curves from blood units in the left heart according to the "concentric" hypothesis.

I. The same as H, according to the "column" hypothesis.

J. to M. Curves which are the resultant of forces generated by the blood in different anatomical positions.

N. Resultant of all forces, theoretical ballistocardiogram according to the "concentric" theory.

O. Resultant of all forces, theoretical ballistocardiogram according to the "column" view.

outlet valves in the manner shown in figure 1, B. In such a case all the units to be ejected, and another to fill the space about the valves, would start together at the beginning of systole. The resulting impacts have been calculated and the curves have been given in figure 1, I.

The minimum possible impacts caused by blood in the heart would occur if the units to be ejected were arranged in more or less concentric layers, as illustrated in figure 1, A. When the heart starts to contract Units 5 and 6 move off in the current, but Units 1, 2 and 3 are out of it for a while. Much of the initial motion of Units 1, 2 and 3 is along opposite radiae, so that little impact results until each unit, leaving position LH 4 in space, begins to enter the current, when it is strongly accelerated. In this arrangement the units to be ejected do not start their longitudinal motion simultaneously but seriatim, and therefore they do not give their impacts at the same time, as in the "column" theory, but in series. In this calculation, the motion of the units before they entered the current was neglected. The results are shown in figure 1, H.

The "concentric" conception seems to us much closer to the truth than the column theory, but nevertheless it may not be quite correct. The centers of mass of the units in the heart cannot be located in the same place. But any tendency to headward movement of the units before they enter the current would be opposed by the downward movement of the base of the heart during systole, so we have no hesitation in neglecting their impacts until the units enter the current.

Summation of impact curves. The families of impact curves, derived from the motion of blood in the aorta, pulmonary artery and heart can now be combined by adding their ordinates at similar intervals of time. Such resultant curves are due to motion of the blood, and motion of the body will be equal and opposite. So these curves must be multiplied by -1 to give the theoretical ballistocardiogram.

Our method of giving numerical value to the coördinates of such a curve for subject Sta. has been given in detail (Starr et al., 1939) and need not be repeated. The present calculation differs in only one particular, in this instance a mass factor of 1 represents the mass of 1 unit of blood (i.e., 10 grams) while in the previous calculation the mass unit represented the output of both sides of the heart, 120 grams. Hence the sums of the ordinate values of our families of curves must be divided by 12 to make them comparable to curve A 4 of figure 5 of our previous paper (Starr et al., 1939). This has been done and the divisions of ordinate in figure 1 correspond to those used in 1939.

Relation of the curve derived theoretically to that recorded. The shape of the theoretical ballistocardiogram (fig. 1, N and O) resembles the normal records. Due to difficulty in placing the zero line the areas of wave I plus wave J are a better index of size than either alone. When the cardiac

impacts are calculated as maximal, using the column assumption, the area of wave I + wave J in the total theoretical impact curve for Sta., is 34 per cent larger than the average found in his ballistocardiogram; if the cardiac impacts are calculated as minimal, using the concentric assumption, the resultant curve is 24 per cent too small. Obviously by combining the two views a theory could be found which would fit our data exactly. But we are inclined to accept the concentric view as approximately correct and other methods of improving the fit have occurred to us. Increasing the mass of blood assigned to the aorta would have this effect, and there is evidence that Bazett's data underestimate the aortic size (Cournand and Ranges, 1941). Also the impacts from peripheral blood have been neglected hitherto. While blood whose longitudinal velocity is retarded by diversion from the descending aorta to the segmental vessels has been calculated to give a small impact, driving the body feetward at the time of the "J" wave, this would surely be overbalanced by the inclusion of impacts from blood in the head, arms, and legs whose sum would provide a headward impact at this time. Assuming that the velocity curve retained its shape in the periphery, we need the impacts of 45 cc. of peripheral blood to secure agreement when the concentric theory is employed. This does not seem unreasonable but we doubt if further speculation is profitable; for the agreement between theory and fact shown by the curves of figure 1, N and O, is as good as we have a right to expect considering the assumptions involved.

We are now in a position to assign parts of the total impacts to the movement of the blood in different anatomical positions and the results are given in figure 1, J to M. By far the largest part of the impacts we record comes from blood while in the aorta, a conclusion similar to that reached independently by Hamilton (1941). The contribution from blood in the pulmonary artery is much smaller. The footward I wave is largely cardiac in origin, the headward J wave is from aortic blood. We have other support for the cardiac origin of the I wave. In figure 3, D, is shown the record obtained from a patient whose heart, because of the operative removal of the left lung, was found by x-ray to be extremely displaced, the apex beat being 4 cm. below the axillary fold. When the diaphragm is up and the heart almost transverse in position, the I wave is hardly detectable. In this situation the cardiac recoil, delivered transversely, is not recorded. The feetward thrust, due to the longitudinal acceleration of the blood turning from the heart into the aorta, occurs throughout systole and so is largely buried in the headward "J" wave.

Changes in our theoretical conceptions. We formerly believed (Starr et al., 1939) that the normal pendulum movements of the cardiac apex might make considerable difference to our records and so be a major source of error in the estimation of cardiac output. We have changed our view

for two reasons. Not only does the blood in the heart itself make a comparatively small contribution to the impacts but the axes of the two ventricles converge towards the midline, so that small pendulum movements while throwing one axis further from the longitudinal would bring the other more into line. Only in extreme pathological displacement of the heart can any abnormality of form be seen in our records which we can attribute to shift in its position.

We previously believed that abnormal dilatations of the aorta would make a major error in our estimation of cardiac output and we were surprised to find that the presence of an aneurism made no recognizable difference in the ballistic records (fig. 3, E). We now see several possible reasons. Clots filling the abnormal lumen would prevent any reduction of velocity. Also short changes of aortic diameter would have little effect, the momentum lost when diameter is increased being regained when it is decreased and vice versa. However, if the aorta entering an aneurism had a different direction from that leaving it an abnormality might be introduced into the record.

Measurements made from x-ray pictures of aortae visualized in the living by intravenous injections of contrast media by Cournand and Ranges (1941) indicate that our estimates of aortic size from Bazett's compilations (1935) of Suter's (1897) autopsy data yield a figure which is somewhat too small. Data obtained from living subjects being infinitely preferable we will change our figures for aortic section area as soon as the newer data are available. The change will have the effect of raising the estimation of cardiac output somewhat and so bring the average of our results obtained on normal persons closer to corresponding data obtained by other methods. While the absolute value of our results will be altered the significance of changes and deviations from the normal will be unchanged.

The effect on the ballistocardiogram of changes in form of the blood velocity curve has been recalculated by assuming a curve in which maximum velocity is attained late in systole, the mirror image of the velocity curve in figure 1, C, and then using the methods described herein. The results look so much like the curves published before (Starr et al., 1939, fig. 5) that they will not be repeated here.

To study the effect of changes in pulse wave velocity we recalculated the impacts of Sta., substituting the pulse wave velocities obtained by Bazett (1941) on an old man: heart subclavian, 5.6; and subclavian to femoral, 11.7 meters per sec. The resulting impact curve could hardly be distinguished from the one in which the average values of his age group, 3.3 and 7.6 meters per sec., were used. So we believe that physiological changes of pulse wave velocity will not make a detectable difference in the ballistic records.

Our first theoretical approximation (Starr et al., 1939) explained the

impacts as the resultant of three curves. The second of these curves was attributed to the arrest of the blood moving headward by the aorta arch and the backward curve of the pulmonary artery. This explanation belongs much better to the second part of the first curve. We now realize that the second and third curves have a common origin; they chiefly represent the impacts due to the movement of the large mass of blood in the descending aorta.

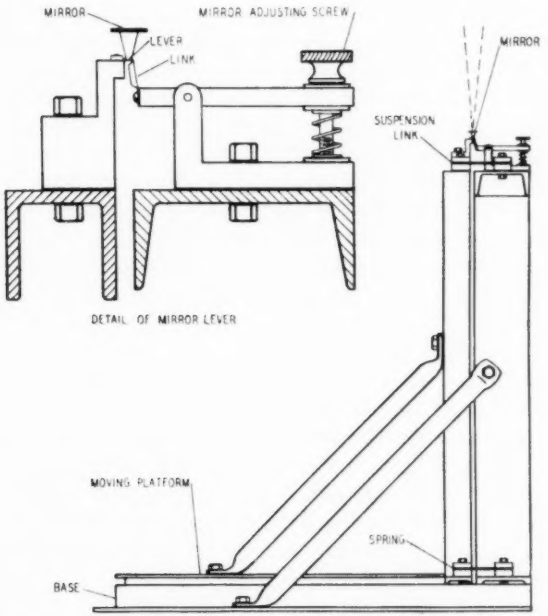


Fig. 2. The construction of the vertical ballistocardiograph with a detail drawing of the adjusting mechanism on the mirror lever.

In spite of these numerous improvements and clarifications in our views, this study supports all the main features of our previous conceptions and we have nothing further to add either to our method of calculating cardiac output from the records or to our interpretation of abnormal forms.

APPARATUS AND RECORDS. As indicated in figure 2 the vertical ballistocardiograph consists of a light weight Duraluminum platform attached to a vertical steel frame which is flexibly connected to a heavy steel base frame, so that the platform can only move in a vertical direction. This vertical movement is restrained by two stiff flat steel springs whose tension can be adjusted by altering the length in use. Relative movement be-

tween the platform and the base frame drives a mirror mounted on a short lever, thus deflecting a light beam. This deflection is recorded on a standard photo-kymograph.

Difficulty was encountered obtaining a record free of serious distortion from vibrations in the building largely due to the elevators. These vertical vibrations ruined the record when the vertical instrument was placed directly on the floor and simple measures such as mounting it on rubber were ineffective. It was necessary to place 500 lbs. of steel plates under the base frame and support the whole on elastic material of diverse properties. One edge is supported by a ridge of rubber-like material 3 cm. wide by 5 cm. thick, the opposite edge by a row of 12 tennis balls compressed hard, the center by about 60 tennis balls compressed lightly. The result is not perfect, for vibrations of a frequency averaging about 13 per second disturb the record repeatedly, for periods of a few seconds each, whenever the elevators are in use.

Calibration. The springs have been adjusted so that 280 grams placed on the platform displaces the light spot image 1 cm. which makes the calibrations of both horizontal and vertical ballistocardiograms the same.

Period of vibration. When weighted with iron and struck a single blow the frequency is as follows: at 100 lbs., 22 vibrations per sec., at 150 lbs., 11.5 per sec., at 200 lbs., 10.5 per sec. Thus the frequency of the vertical instrument is almost 80 per cent faster than the horizontal at 100 lbs., but only about 10 per cent faster at 200 lbs.

Vibrations in the vertical human body. Thirteen subjects, standing on the platform, were struck a series of taps on the head or shoulder. Analysis of those records which chanced to fall between cardiac complexes showed that the vibrations set up had an average frequency of 5.57 per sec. But the range was from 5 to 6.7 per second in individual subjects and this average is not significantly different from 5.72 per sec., the corresponding average found in horizontal subjects.

After such taps had deflected the light spot, on its return it overshot the base line by an amount which averaged 65 per cent of the height of the previous deflection. This is significantly different from the corresponding average of 40 per cent found in horizontal subjects. The body provides more damping in the horizontal than in the vertical position.

Taking records. As subjects depress the platform a different amount depending on their weights, the mirror adjusting screw must be used to direct the light spot to the camera after each change of subject. Then the operator watches the flickering image, chooses a time when distortion is at a minimum and takes the photograph.

Measurement and calculation from the records. The area method (Starr et al., 1939) has been used to calculate cardiac output. Typical large and small complexes of the respiratory cycle were selected. The line on the

record has considerable width and in making all measurements we used the top edge. A base line was drawn at the position this edge would occupy if the heart were not beating. With this base line waves I and J formed areas which were almost triangular, the first below, the second above the base line. With a ruler and sharp pencil true triangles were superimposed, the aim being to shave off from the sides as much area as was added at the apex. The bases of these constructed triangles were measured in fractions of a second, their altitudes in millimeters. The values obtained in the two selected complexes were averaged and cardiac output was estimated as described before (Starr et al., 1939).

Statistical methods. The methods of Fisher (1938) were employed. The word significant is always used in the statistical sense, indicating a probability of 0.05 or less that the difference is due to chance.

RESULTS. *Comparison of ballistocardiograms of vertical and horizontal subjects.* Typical "V" records are shown in figure 3 and they show systolic complexes of a smaller amplitude than the corresponding "H" records. This is in accord with the diminished stroke volume found in the erect posture by all other cardiac output methods. The time relations to the electrocardiogram are the same in "V" and "H" records (fig. 3, C).

In normal subjects the systolic complexes have a *similar form* in both "H" and "V" records. To investigate the finer details, the records of 20 subjects tested in both positions were chosen at random; the average durations of the "H" and "V" downward deflections, I waves, were 0.058 and 0.056 sec. respectively; the upward deflections, J waves, 0.091 and 0.096 sec., differences well within the error of placing the base line.

Nevertheless *major differences* usually permit the recognition of "V" records at a glance. Due to shifting of the subject's weight from foot to foot the base line may wander. Deflections in diastole are often higher in relation to the systolic complexes, so that the latter do not stand out as clearly as in "H" records. When the elevators start or stop rapid vibrations at a frequency averaging 13.5 and ranging from 10 to 15 per second, often confuse the "V" records for several seconds. Interfering vibrations of this frequency are seldom, if ever, seen in "H" records. Muscle tremors, brought out by the use of the muscles in standing, may confuse or destroy the usefulness of the "V" record, while the "H" record is unaffected (fig. 3, I).

EXPERIMENTS. *Subjects.* The normal subjects used were either medical students, young doctors, or hospital and laboratory technicians. There were 36 men and 22 women all over 18 and under 35 years of age except the first author. No test was made within 2 hours of a meal.

Technique. The subject lay relaxed on the horizontal table for 15 minutes or longer. At the end of this period blood pressure and ballistocardiogram were taken. He then arose and took several steps to the vertical

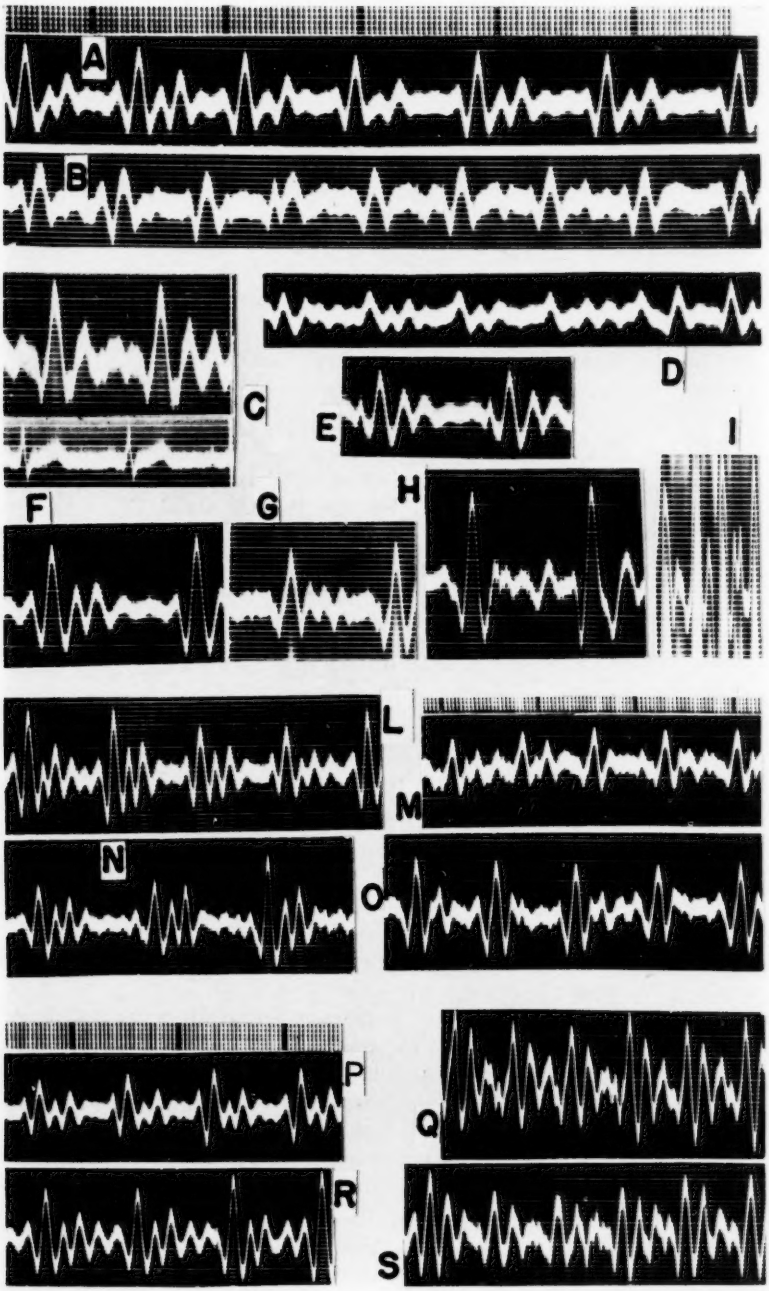


Fig. 3

ballistocardiograph which stood at the foot of the horizontal instrument, and stepped up 9 inches to its platform, on which he stood relaxed with his feet either together or apart, records being taken 1 and 2½ minutes after taking his stance in most experiments, with additional observations at 5, 10 and 15 minutes in a few. Prolonged standing was not studied.

Fig. 3. Ballistocardiograms of subjects in the vertical and horizontal positions.

A. to I. Reproductions approx. actual size. Time record over A applies to all. Largest interval 1 sec.

A. Subject M. L., age 34, 5 ft. 3 in., 124 lbs. A normal woman after 15 min. rest horizontal.

B. Same after standing 2½ min.

C. Vertical ballisto. of H. E., age 24, 5 ft. 11 in., 158 lbs. Male. Lead 1 of the electrocardiogram recorded simultaneously on the same film.

D. Horizontal ballisto. of E. W., age 28, 5 ft. 4 in., 114 lbs. Female. Six months after left pneumonectomy. X-ray shows the heart in contact with the left chest wall; the apex impulse is in the axilla. Note absence of initial downward deflection (I wave) in smaller complexes.

E. Horizontal ballisto. of R. M., age 58, 5 ft. 8 in., 133. Male. Large aneurysm of ascending and transverse aorta. Note normal record in spite of the huge dilatation of the aorta.

F. S. D., 24, 5 ft. 6 in., 132 lbs. A normal medical student after resting 15 min. horizontal. B.P. 112/82.

G. Same after standing the next 2½ min., B.P. 114/90.

H. Same lying 10 minutes after receiving 0.5 cc. adrenalin s.c. B.P. 140/68.

I. Same standing next 2½ min. Note that the adrenalin tremor destroys the vertical record while the horizontal is affected little if at all.

L. to O. Effect of Paredrine. Records reduced to two-thirds actual size. Time record over M applies to all this group. Subject E. W., normal medical student, age 23, 6 ft. 1 in., 155 lbs.

L. After 15 min. rest horizontal. Cardiac output per minute 26 cc. per min. per lb. body weight, + 13 per cent of average normal. B.P. 120/80.

M. Same subject after standing 2½ min. C.O. = 24 cc. B.P. 122/88.

N. Same subject lying ½ hr. after receiving 20 mgm. N-methyl paredrine hydrochloride subcutaneously. C.O. = 21. B.P. 168/110.

O. Same subject after standing next 2½ min. C.O. = 29 cc. B.P. 146/96. Note that after paredrine the response of the cardiac output to arising was reversed.

P. to S. Effect of an abdominal binder. Records reduced to three-quarters actual size. Time record over P applies to all this group. Patient W. T., age 50, 5 ft. 6 in., 128 lbs. Complains of frequent attacks of faintness when standing.

P. After 15 min. rest. Cardiac output = 28 cc. per min. per lb.; + 22 per cent of average normal. B.P. 90/60.

Q. After standing next 2½ min., feeling dizzy and faint. C.O. = 40 cc. B.P. 88/70.

R. After 15 min. rest with abdominal binder in place. C.O. = 34 cc. B.P. 95/65.

S. After standing next 2½ min. with binder. No symptoms. C.O. = 33 cc. B.P. 95/75. A vibration in the building affects the center of this record. Note that dizziness occurred in the presence of an abnormally large cardiac output per minute and was absent when this was smaller. Also the presence of the binder changed the response of the cardiac output to arising.

The plateau after arising. Records started as soon as the subject reached the platform showed diminishing impacts for a brief period which we interpreted as the effects of the exertion passing off. After one minute of standing a plateau was attained, for the averages show no significant difference for the next 10 minutes in the 3 subjects tested (table 1). However, after 15 minutes' standing the averages showed a very slight but significant increase in cardiac output in these three subjects that may well be correlated with the increased restlessness often seen at this time. Duplicate estimations made in every experiment at 1 and $2\frac{1}{2}$ minutes, or $2\frac{1}{2}$ and 5 minutes, after assuming the erect posture confirmed the impression that

TABLE 1

Changes in cardiac output per minute during 15 minutes' quiet standing

DATE	SUBJECT	SEX	AGE	CHANGE FROM VALUE FOUND AFTER $2\frac{1}{2}$ MINUTES' STANDING		
				5 minutes	10 minutes	15 minutes
				per cent	*per cent*	*per cent*
4-19-40	Sta.	M	45		−5	+5
4-29-40				+9	+4	+16
6- 3-40				0	+5	+5
1-21-41				−10	+4	+4
4-24-40	C. H.	F	26	−7	+4	−11
5- 1-40				−18	−11	+11
5-10-40				+15	+30	+15
5-31-40				−5	+10	+20
12-24-40	M. T.	F	25	−5	−5	−10
1-20-41				+5	−10	+5
Averages				−2	+2.6	+6
Significant for P = 0.05				No	No	Yes

the circulation was steady during this period. As records taken at $2\frac{1}{2}$ minutes are common to all experiments, only these results will be reported.

Changes in the circulation on arising. Fifty-six subjects performed the test for the first time and the results are recorded in table 2. The changes of pulse rate and blood pressure which occurred are so familiar that only the averages have been given in table 2. The average cardiac output per beat diminished significantly; the average cardiac output per minute was unchanged.

The average of these results contrasts with those obtained on the first author and his two technicians in tests made at monthly intervals (table 3). The latter two subjects showed the same cardiac output per minute stand-

TABLE 2

Relation of cardiac output per minute after resting 15 minutes recumbent and after standing the next 2½ minutes at rest

First test on each subject. Cardiac output given in per cent deviation from average normal which equals 51 cc. per min. per kilo body weight, or 23 cc. per min. per lb., when Bazett's data (1935) on aortic size are used. Normal limits ±22 per cent.

SUBJECT	SEX	CARDIAC OUTPUT PER MINUTE		SUBJECT	SEX	CARDIAC OUTPUT PER MINUTE		SUBJECT	SEX	CARDIAC OUTPUT PER MINUTE	
		When horizontal	Change on standing			When horizontal	Change on standing			When horizontal	Change on standing
		per cent deviation from normal average	*per cent*			*per cent deviation from normal average*	*per cent*			*per cent deviation from normal average*	*per cent*
Ho.	M	+35	−10	Sy.	F	+4	−13	Lo.	M	−9	−9
Cal.	F	+22	−5	Ro.	M	+4	−12	Co.	M	−9	+14
Cr.	M	+22	+11	Am.	M	+4	−4	Cap.	M	−9	+14
We.	M	+17	−4	La.	M	+4	0	Al.	M	−9	+19
Ha.	M	+17	0	Do.	M	+4	+8	Koe.	M	−9	+38
Ho.	F	+17	+4	Jo.	F	0	−17	Ros.	M	−13	−20
Br.	F	+17	+4	He.	M	0	−17	Ki.	M	−13	0
Ja.	M	+17	+11	To.	F	0	+4	Ze.	F	−17	−4
Ji.	F	+13	−15	Erb.	M	0	+4	Calk.	F	−17	+4
Ev.	F	+13	−15	Po.	M	0	+13	Ca.	M	−17	+16
Con.	M	+13	−12	Fr.	M	0	+22	Ul.	M	−17	+5
Wi.	M	+13	−8	Bra.	M	0	+22	Ir.	F	−17	+10
Er.	M	+13	+4	De.	F	−4	−25	Ha.	F	−22	0
Fra.	F	+13	0	Vi.	M	−4	−18	Ka.	M	−22	+6
El.	F	+9	−32	Ko.	F	−4	+15	Na.	M	−22	+11
Ir.	M	+9	−16	Mc.	F	−4	+27	Ba.	F	−22	+17
My.	M	+9	−18	Be.	F	−4	+14	Ri.	M	−22	+17
Mu.	F	+9	−12					La.	M	−30	+6
Pa.	F	+9	+8								
Iro.	M	+9	+16								
Pas	M	+9	0								

Average changes on assuming the erect position. Means and standard deviations about the means

	MEANS	STANDARD DEVIATION	SIGNIFICANCE		MEANS	STANDARD DEVIATION	SIGNIFICANCE
					mm. Hg	*mm.* Hg	
Cardiac output per minute....	+1%	14%	No	Systolic B.P.....	+1.1	7	No
Stroke volume..	−17.6%	9.6%	Yes	Diastolic B.P.....	+6.4	8	Yes
Pulse rate.....	+16.6 per min.	8.3 per min.	Yes				

TABLE 3

Relation of cardiac output per minute after resting 15 minutes recumbent and after standing the next 2½ minutes

Repeated tests in single subjects none of whom were originally accustomed to the test, except Sta.

SUBJECT	DATE	CARDIAC OUTPUT PER MINUTE		SUBJECT	DATE	CARDIAC OUTPUT PER MINUTE		SUBJECT	DATE	CARDIAC OUTPUT PER MINUTE	
		When horizontal	Change on standing			When horizontal	Change on standing			When horizontal	Change on standing
		per cent deviation from normal average	*per cent*			*per cent deviation from normal average*	*per cent*			*per cent deviation from normal average*	*per cent*
Sta.	4–19	+17	−19	Be.	2– 4	−4	+14	Calk.	3–14	−17	+4
	4–23	+13	−10		2–18	−4	0		3–28	−9	−12
	4–29	+22	−18								
	6– 3	+9	−12	Ha.	2– 7	−22	0	Pa.	3–21	+9	+8
	7–15	+4	−21		2–17	−25	+8		3–25	0	−6
	11–18	+17	−18								
	12–24	+9	−12	Ze.	2–10	−17	−4	Mu.	3–21	+9	−12
	1–21	+4	−13		2–18	−25	−6		3–25	+30	−24
	2–27	+22	−11								
	3–21	+30	−13	Jo.	2–11	0	−17	Li.	3–22	−9	+31
					3–17	−39	0		3–24	0	−9
Ho.	4–24	+17	+4								
	5– 1	+29	−7	Ba.	2–14	−22	+17	De.	3–24	−4	−25
	5–10	+24	−17		2–27	−13	−5		4– 1	−9	−14
	5–31	+25	−20								
	6– 4	+25	+8	Sy.	2–28	+4	−13	Ir.	3–27	−17	+10
					3–11	−17	+5		4– 1	−13	0
To.	11–19	0	+4								
	12–24	+13	−19	Cal.	3– 1	+22	−5	Ji.	4–22	+13	−15
	1–20	+22	−29		3–15	+26	+14		4–28	+13	+4
	2–27	+9	−8								
	3–21	0	−14	Ri.	3–10	−22	+17	El.	4–22	+9	−32
					3–20	−13	+10		4–28	+9	−12
				Ko.	3–11	−4	+15				
					3–27	−4	+5				

Average change on standing, first test . . . +2% Difference not significant
Average change on standing, second test. −5%

ing as lying the first time they underwent the experiment but on most subsequent tests they exhibited a smaller circulation when erect. The first author, a veteran subject, always showed a smaller circulation when

erect. Therefore we suspected that slight emotion incident to the performance of the test for the first time might play a part in the results.

Accordingly 20 subjects who had had no previous experience on the apparatus were given 2 tests about a week apart and the results are recorded in table 3. The average change in the cardiac output per minute, on assuming the erect position, was +2 per cent at the first test and −5 per cent on the second, a difference in the direction expected but not of statistical significance.

Agents altering the response to arising. In the course of class demonstrations of the action of drugs in normal medical students, records were obtained at the end of alternate periods of lying 10 or 15 minutes, and standing $2\frac{1}{2}$ minutes, both before and during drug action. Sixty such experiments were made. The striking feature of the results was the demonstration that in certain types of drug action the circulation in the horizontal position might be affected in one direction, while in the vertical position this effect was reversed. The most striking examples occurred in experiments after n-methyl paredrine hydrochloride[1] and one series of such records is given in figure 3. At a time when the drug had caused some slowing of the pulse rate and consequent diminution of cardiac output per minute when the subject was horizontal, as found by Altschule and Iglaver (1940), the circulation was strongly stimulated when he stood erect. This effect was obtained on all 8 subjects given the drug, and the accelerated circulation in the erect posture showed no tendency to diminish for 10 minutes after arising; longer periods were not tested.

Most of the drugs tested affected the circulation similarly in the two positions, and while a diverse effect was seen occasionally it was never of the degree or with the consistency seen after paredrine. Therapeutic doses of adrenalin regularly increased the circulation in both positions, but in one subject as its action was passing off, the increase was found greater in the horizontal position. Eight milligrams benzedrine caused a similar effect on one occasion. Histamine gave inconsistent results; on one occasion 0.35 mgm. increased the circulation when the subject lay without affecting it as he stood, while on another 0.4 mgm. increased it in both positions. Nitroglycerine gr. $\frac{1}{150}$ under the tongue always increased the circulation in both positions. Therapeutic doses of strychnine, metrazol, coramine, atropine, pitressin, and prostigmin, and distilled water, all given subcutaneously, had no noteworthy effect on the amount of the circulation in either position.

If the abdominal wall is relaxed the application of a binder often increases the horizontal circulation but diminishes the vertical, as is shown

[1] We are indebted to the Smith, Kline, and French Laboratories for the drug employed.

in figure 3. But in most normal subjects a binder causes little change in either position.

Symptoms of faintness and the general circulation. On six occasions normal subjects complained of transient symptoms of faintness, lightheadedness, or dizziness while standing on the vertical ballistocardiograph. In three instances the symptoms occurred spontaneously, in the remainder they occurred during drug action, especially after nitroglycerine placed under the tongue. In no case was the cardiac output smaller during the symptoms than in corresponding periods when the patient was symptom free. On the contrary, the symptoms were often experienced during a period in which the cardiac output was definitely greater than that existing when they were absent (fig. 3, Q). A slight diminution of blood pressure was usually, but not always observed during these symptoms, but in no case was the remaining pressure insufficient to raise blood to the top of the head. Schneider and Crampton (1934) and also Schellung and Heinemeier (1933) reported similar findings and these results were expected by Starr and Collins (1931b).

Discussion. The ballistocardiograph is not to be regarded as an instrument of high accuracy for the estimation of cardiac output. Nevertheless the general agreement of the results with those obtained by other methods has been confirmed (Cournand and Ranges, 1941). In the light of 5 years' experience the internal evidence continues very good; i.e., duplicates agree well, and agents known to increase cardiac output, such as exercise and certain drugs, increase the size of the impacts invariably. The method gives most reasonable results; that it is an easy qualitative measure of changes of cardiac output cannot, in our opinion, be disputed; and this is sufficient justification for its use. But its possibilities are far greater than this because, by means of Newton's "Laws of Motion," the record is mathematically related to fundamental cardiac functions, the amount of blood ejected and the manner of its ejection. Assumptions are necessary to estimate this relationship; it is our hope that further work will increase our knowledge of them. The absolute quantitative accuracy of this, and the other cardiac output methods, is unknown.

The number of assumptions necessary varies with the nature of the experiment. Thus to compare the cardiac output of different subjects the size of their great vessels must be estimated; to compare the cardiac output of the same subject under different conditions, as in this investigation, this is not necessary.

Cardiac outputs estimated by ballistocardiograms can be directly compared only if the manner of ejecting blood from the heart, i.e., the curve of blood velocity during ejection, remains normal. We have evidence (Starr et al., 1938) that changes in the form of this curve would alter the form of the ballistic record so that the presence of this abnormality could

be recognized. No such changes occurred during these experiments, although distortions of form, similar to those calculated from abnormal blood velocity curves (Starr and Schroeder, 1940) are common in persons with damaged hearts.

Our vertical ballistocardiograph is distinctly inferior to our horizontal instrument. The former is much more difficult to insulate from vibrations due to the 3 elevators in our steel and concrete building; more primitive housing might obviate this difficulty in other places. Its record is much more likely to be completely ruined by muscular tremors. The advantage of the horizontal position was appreciated by Gordon (1877) who seems to have been the originator of this field (Lamport, 1941). The investigations of Abramson (1933) and Heald and Tucker (1922) must have been handicapped by the fact that they used vertical instruments.

Some types of investigation cannot be performed. Patients subject to fainting placed on the platform have developed uncontrollable muscular movements which ruined the record long before any symptoms set in. Patients who are weak from any cause, or who have been long in bed, usually can not stand still enough to give usable "V" records, although their records when horizontal may be entirely satisfactory. Nevertheless satisfactory "V" records can be obtained on almost all normal persons and on a majority of ambulatory patients.

The average relation between the cardiac output lying and after standing 15 minutes or longer has been disputed, some authors finding a diminution, others no change (McDowell, 1938). The same discrepancy appears in our results for, while our averages show no change, the circulation of certain subjects as Sta. (table 3) regularly diminishes in the erect posture. Our attempt to explain the difference as the result of becoming accustomed to the test yielded suggestive evidence, but failed to prove the point, so we sought for other explanations. Another factor became obvious as soon as the results were arranged in order of magnitude as in table 2. Subject Sta. is one of many whose cardiac output per minute, when lying, was above the average normal, and who showed a conspicuous diminution of the circulation on arising. With few exceptions it is those whose cardiac output per minute, when recumbent, is below the normal average who show no change, or an increase, when they arise. There is significant correlation between the original level of the cardiac output when lying and the change on arising.

The search for similar correlations in the literature was handicapped by the failure of a number of authors to report the weight of their subjects. In our data the relation is significant only when weight is taken into account. Nevertheless, in the data of Fisher (1932) and Donal, Gamble and Shaw (1934) both the absolute value of the cardiac output per minute, and the cardiac output per minute related to body weight, showed

significant correlation with the change on arising, so we have not disregarded the data when weight was not given.

In table 4 the results of 4 authors using the same method are arranged in order of magnitude. The strong positive correlation between the level

TABLE 4

Data on the change of cardiac output in lying and standing subjects, obtained from McMichael (1937); Schneider and Crampton (1934); Grollman (1932); and Goldbloom, Krause and Lieberson (1940); and arranged in order of magnitude of cardiac output when lying

AUTHORS	SUBJECT'S CARDIAC OUTPUT LYING	CHANGE ON STANDING	AUTHORS	SUBJECT'S CARDIAC OUTPUT LYING	CHANGE ON STANDING	AUTHORS	SUBJECT'S CARDIAC OUTPUT LYING	CHANGE ON STANDING
	liters per minute	*per cent*		*liters per minute*	*per cent*		*liters per minute*	*per cent*
S & C	6.5	−22	G.	4.3	−19	G. K. L.	3.8	+9
McM.	5.8	−33	G.	4.3	−16	G. K. L.	3.8	+3
McM.	5.3	−21	G.	4.3	−9	McM.	3.7	+30
S & C	5.2	−29	G.	4.3	−2	G. K. L.	3.6	+5
S & C	5.0	−26	S & C	4.2	−10	G.	3.5	0
McM.	5.0	−14	G.	4.1	−7	McM.	3.5	0
McM.	4.9	−25	G.	4.1	0	G.	3.2	0
McM.	4.8	+2	G. K. L.	4.1	+4	G.	3.2	+3
McM.	4.7	−34	G.	4.0	−5	McM.	3.0	0
S & C	4.4	−18	G. K. L.	4.0	0	McM.	2.6	+4
McM.	4.3	−23	McM.	3.9	−3	G.	2.4	+4

TABLE 5

Relationship between the magnitude of the cardiac output per minute with the subject recumbent and the change on arising in per cent of the recumbent value

AUTHORS	NUMBER OF TESTS	CARDIAC OUTPUT ABSOLUTE VALUE, OR REFERRED TO BODY WEIGHT	LEVEL BELOW WHICH CORRELATION IS NOT SIGNIFICANT FOR P = 0.05	CORRELATION COEFFICIENT
Starr and Rawson (this paper)	56	body wt.	0.26	0.30
Donal, Gamble and Shaw	23	body wt.	0.40	0.42
Fisher	47	body wt.	0.28	0.57
4 Authors of table 4	33	abs.	0.33	0.69
Schellong and Heinemeier	28	body wt.	0.36	0.41
Nylin	11	body wt.	0.55	−0.18
Bock	17	abs.	0.45	0.46

of cardiac output of the recumbent subjects and the change on standing is obvious at a glance.

The statistical results are given in table 5. With the exception of a short series by Nylin (1934), all the series long enough to make statistical

analysis worth while, and obtained by cardiac output methods still in use, demonstrate the correlation mentioned above. But in the absence of a statistical analysis the fact has not been realized.

This conception resolves some of the discrepancies in the literature for the results of Schneider and Crampton (1934), believed to be at variance with those of Grollman (1932) because the averages were different, are now seen to fit well with the other data when its larger relations are discovered. Most of Grollman's (1932) subjects were in the basal condition; none of Schneider and Crampton's (1934) were basal, so the resting cardiac output was larger in the latter investigation.

Thus some of the diversity of recorded result can be explained by the experimental conditions employed or by chance in the selection of subjects, but there are surely other factors. Most of these studies differ from ours in that, since estimation of cardiac output by the methods previously available required considerable time, the interest necessarily centered on the effects of standing for longer periods than we usually employed. Prolonged standing provides time for the accumulation of blood and lymph in the dependent parts of the body and so brings into play a slow physiological mechanism not important in the majority of our experiments (Asmussen et al., 1939).

Other errors must be present, for cardiac output methods are crude. The contrasting experiences of Stewart and Cohn (1932) and Harrison (1939); and of Grollman (1932) and Gladstone (1935); and the evidence of Sweeney and Mayerson (1937), indicate that small differences in the duration of rebreathing can make no little difference in the results of those methods which aim to secure gaseous equilibrium in the lungs before the blood returns a second time. So experimental error and slight differences in experimental technique are surely additional factors in the diversity of the recorded results. Nevertheless, the conception cited above does much to unify the field and the divergent results do not occur more frequently than one could expect from chance accumulation of the inherent errors.

The published data suggest that there is a limit below which the cardiac output per minute of a standing subject cannot go with safety, and that this limit is located near the middle of the normal range found in recumbent subjects, whose circulations are governed by different and less rigid requirements. If the cardiac output per minute of the recumbent subject exceeds his standing requirement, then the circulation usually diminishes on arising. If the recumbent value does not exceed the standing requirement, there is no change or an increase in the circulation when the subject arises.

Our results show that drugs and other physiological agents acting on the circulation do not necessarily produce the same effect when the subject is lying as when he is standing and so may profoundly modify the response

to arising. Data on the action of such agents in the erect posture is almost non-existent and the apparatus herein described seems well suited to obtain more information.

SUMMARY

Theoretical studies to account for the origin of the ballistic waves have been continued. The major part of the systolic complex is due to the movements of the blood in the aorta.

A ballistocardiograph, designed to secure records in standing or sitting subjects, has been constructed.

By this means we have studied the immediate response of the circulation to change of position, concentrating our attention on the plateau which extends from 1 to 10 minutes after arising.

In normal persons the average cardiac output per minute is the same at this time as before they arose.

On assuming the erect posture, the cardiac output per minute of some subjects regularly diminishes, in others it remains the same, in others it increases somewhat. The original state of the circulation is an important factor in this difference, i.e., when the resting cardiac output is in the upper half of the normal range, it diminishes on arising in the majority of tests; if in the lower half of the normal range, it increases on arising in the majority.

The drug N-methyl paredrine hydrochloride, which diminishes the cardiac output per minute in the supine position, increases it in the erect position, so that the response to arising is profoundly altered. No other drug tested gave effects comparable to this.

The application of an abdominal binder, in certain persons, affects the circulation in different directions in the erect and supine positions.

Symptoms of dizziness or lightheadedness on arising were not coincident with a diminished output of the heart.

REFERENCES

Abramson, E. Skand. Arch. Physiol. **66:** 191, 1933.

Altschule, M. D. and A. Iglauer. J. Clin. Investigation **19:** 497, 1940.

Asmussen, E., E. H. Cristensen and M. Nielsen. Skand. Arch. f. Physiol. **81:** 214, 1939.

Bazett, H. C., F. S. Cotton, L. B. LaPlace and J. C. Scott. This Journal **113:** 312, 1935.

Bazett, H. C. In Macleod's Physiology in modern medicine. C. V. Mosby Company, St. Louis, 1941.

Bock, H. E. Ztschr. f. d. ges. exper. Med. **92:** 782, 1934.

Cournand, A. and H. A. Ranges. This Journal **133:** P251, 1941.

Donal, J. S., Jr., C. J. Gamble and R. Shaw. This Journal **109:** 666, 1934.

Fisher, J. L. Arbeitsphysiol. **6:** 112, 1932.

Fisher, R. A. Statistical methods for research workers. Oliver and Boyd, London, 1938.

GLADSTONE, S. A. This Journal **112:** 705, 1935.
GOLDBLOOM, A. A., M. L. KRAMER AND A. LIEBERSON. Arch. Int. Med. **65:** 178, 1940.
GORDON, J. W. J. Anat. and Physiol. **11:** 533, 1877.
GROLLMAN, A. The cardiac output of man in health and disease. C. C. Thomas, Baltimore, 1932.
HAMILTON, W. F. This Journal **133:** P313, 1941.
HARRISON, T. R. Failure of the circulation. The Williams and Wilkins Company, Baltimore, 1939.
HEALD, C. B. AND W. S. TUCKER. Proc. Royal Soc. Series **B93:** 281, 1922.
LAMPORT, H. Science **93:** 305, 1941.
MACHELLA, T. E. This Journal **115:** 632, 1936.
McDOWELL, R. J. S. The control of the circulation of the blood. Longmans, Green and Company, New York, 1938.
McMICHAEL, J. Quart. J. Exper. Physiol. **27:** 55, 1937.
NYLIN, G. Skand. Arch. Physiol. **69:** 237, 1934.
ROESLER, H. Atlas of cardioroentgenology. C. C. Thomas, Baltimore, 1940.
SCHELLONG, F. AND M. HEINEMEIER. Ztschr. exper. Med. **89:** 49, 1933.
SCHNEIDER, E. C. AND C. B. CRAMPTON. This Journal **110:** 14, 1934.
STARR, I. AND L. H. COLLINS, JR. This Journal **96:** 228, 1931a.
J. Clin. Investigation **9:** 561, 1931b.
STARR, I., A. J. RAWSON, H. A. SCHROEDER AND N. R. JOSEPH. J. Clin. Investigation **17:** 506, 1938.
This Journal **127:** 1, 1939.
STARR, I. AND H. A. SCHROEDER. J. Clin. Investigation **19:** 437, 1940.
STEWART, H. J. AND A. E. COHN. J. Clin. Investigation **11:** 917, 1932.
SUTER, F. Arch. f. path. Anat. u. Pharmakol. **39:** 289, 1897.
SWEENEY, H. M. AND H. S. MAYERSON. This Journal **120:** 329, 1937.

DESOXYCORTICOSTERONE AS A PROPHYLACTIC FORE-TREATMENT FOR THE PREVENTION OF CIRCULATORY FAILURE FOLLOWING HEMORRHAGE AND SURGICAL TRAUMA IN THE ADRENALECTOMIZED DOG[1]

W. M. PARKINS, W. W. SWINGLE, J. W. REMINGTON AND V. A. DRILL

From the Section of Physiology, Biological Laboratory, Princeton University

Accepted for publication June 30, 1941

The synthetic adrenal steroid desoxycorticosterone acetate (D.C.A.) will, in small doses, adequately maintain adrenalectomized animals (1, 2, 3). It will also protect the dog lacking adrenals against circulatory collapse induced by *a*, muscle trauma; *b*, intraperitoneal injection of isotonic glucose; *c*, infusion of massive doses of epinephrine (4). According to Perla *et al.*, D.C.A. and saline protects normal rats against histamine (5). However, it does not prevent the circulatory failure which follows intestinal stripping in the adrenalectomized dog (4).

Selye, Dosne, Bassett and Whittaker (6) and Weil and Browne (7) have reported negative results when D.C.A. was employed in attempts to prolong the survival of intact rats and rabbits subjected to intestinal trauma. Selye and Dosne (8) state that corticosterone, which differs rom D.C.A. by the presence of a hydroxyl group at C_{11}, was effective, and advocated its use in the treatment of traumatic shock in man. The writers (4) have found corticosterone to be an efficient agent in preventing the circulatory collapse resulting from intestinal manipulation in adrenalectomized dogs.

D.C.A. was used in this study.[2] The two procedures chosen to produce circulatory collapse were of qualitatively different nature. One involved the physiological responses to a simple loss in circulating blood volume, i.e., massive hemorrhage; the other, surgical trauma incident to a single stage bilateral adrenalectomy. In the latter procedure blood loss is negligible, but trauma to a not inconsiderable amount of nervous tissue in the immediate vicinity of the adrenal glands is often unavoidable.

METHODS. In the hemorrhage experiments trained, unanesthetized dogs

[1] Part of the expenses of this investigation was defrayed by Julian M. Livingston of New Rochelle, N. Y.

[2] We are indebted to the Ciba Pharmaceutical Products, Inc. for generous supplies of the desoxycorticosterone acetate (Percorten) used in these experiments.

were bled at an approximately uniform rate of 10 cc. blood per minute, continued until the arterial pressure had been reduced to 50–40 mm. Hg. The blood was taken by needle-puncture from the femoral artery, using syringes inserted into a three-way stopcock, with the side arm connected to a fluid trap and sphygmomanometer (9). By merely rotating the stopcock, arterial pressures could be taken at will during the course of the hemorrhage.

The single stage bilateral adrenalectomies were performed under nembutal anesthesia. In certain of these operations, and previous to dissection of the glands for removal, the nerves in the region of the adrenals were locally blocked by means of a 4 per cent solution of procaine hydrochloride in sterile water, prepared from Novocaine (Metz) crystals.

The D.C.A. treated animals were given 20 mgm. (four 5 mgm. doses intramuscularly at 24, 18, 12 and 2 hrs.) prior to the experiment.

The techniques used in blood chemical analyses and arterial pressure determinations have been described elsewhere (10).

I. *The effectiveness of D.C.A in increasing the resistance of the adrenalectomized dog to hemorrhage.* The intact, unanesthetized dog, can withstand the loss of 40 to 54 cc. of blood per kgm. body weight before the blood pressure declines permanently to shock levels (11). If the bleeding is stopped at any stage previous to this, rapid blood dilution and a steady rise in pressure follows. The adrenalectomized dog not receiving extract, still eating full rations, and without obvious signs of physical weakness, but with the arterial pressure lowered by some 20 mm. Hg from normal, cannot withstand the loss of 4 to 8 cc. blood per kgm. body weight without showing an abrupt pressure fall to shock level. In this type of experimental animal, and in sharp contrast to the intact dog, the blood pressure will not spontaneously rise from this level, and death invariably follows within a few hours unless extract treatment is given (11).

The animals subjected to hemorrhage consisted of ten vigorous adrenalectomized dogs: four D.C.A. treated, and six controls. Two of the controls, when bled, were receiving adequate but minimal maintenance daily doses of cortical extract. Four animals served as controls of a somewhat different type, since extract therapy had been withdrawn for a period of 24 hours prior to hemorrhage. During this interval negligible changes had occurred in blood chemistry, body weight, vigor and blood pressure. The blood urea nitrogen was in general slightly elevated, however (table 1).

The blood pressure changes during the course of the hemorrhage in a representative D.C.A. treated animal, and in one from which extract therapy had been discontinued for 24 hours, are shown in figure 1. The first withdrawal of blood was followed, in the D.C.A. treated dog, as in the intact one, by a rise in arterial pressure. This rise was sustained until

some 20 to 28 cc. per kgm. body weight had been withdrawn. The pressure then showed a rapid decline, so that the removal of only 5 to 12 cc. per kgm. body weight additional blood sufficed to produce a lowering to shock levels. In this particular dog, the pressure was normal or above throughout 83 per cent of the total hemorrhage, and then fell 70 mm. Hg with the final 17 per cent.

The dogs receiving maintenance doses of cortical extract showed a much shorter period than the D.C.A. treated animal, during which the pressure was elevated above normal. Thus, only about 10 cc. blood per kgm. body weight could be removed before the pressure fell below normal. An additional 6 cc. lowered it to shock levels. The dogs from which extract had been withheld for 24 hours showed no initial rise in pressure (fig. 1).

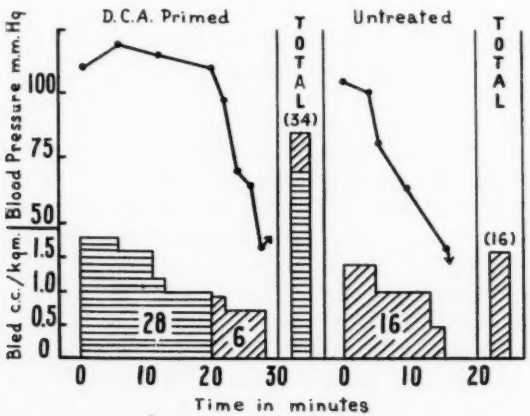


Fig. 1. Effect of hemorrhage in D.C.A. primed adrenalectomised dog

In these animals, therefore, the pressure was falling over the whole course of the hemorrhage. A pressure of 40 mm. Hg was produced by the removal of only half as much blood as that required by the D.C.A. treated dog. Arteriolar capacity adjustments appeared to be lacking in the animals lacking hormone reserves.

Following completion of the hemorrhage, the intact animals, the adrenalectomized dogs given fore-treatment with D.C.A., and those receiving large amounts of cortical extract, all showed full recovery of the arterial pressure to normal levels with disappearance of all symptoms. Two of the four D.C.A. treated dogs recovered within 7 hours following the bleeding. The other two animals exhibited an immediate pressure rise of 22 to 24 mm. Hg and then suffered a partial relapse for 4 to 5 hours, later recovering completely in 36 to 48 hours without additional treatment of any kind.

Dogs receiving merely maintenance doses of cortical extract may show,

on cessation of the bleeding, a spontaneous rise of blood pressure varying from 10 to 20 mm. Hg. However, the rise was not sustained and a regression invariably followed, leading to death from circulatory collapse

TABLE 1

Blood studies of adrenalectomized dogs subjected to hemorrhage

DATE	TIME	BLOOD PRESSURE	PULSE	HEMATOCRIT	HEMOGLOBIN	SERUM SODIUM	SERUM CHLORIDE	SERUM POTASSIUM	BLOOD UREA NITROGEN	BLOOD SUGAR	REMARKS
		mm. Hg	*per minute*	*per cent*	*grams per cent*	*m-eq. per l.*	*m-eq. per l.*	*m-eq. per l.*	*mgm. per cent*	*mgm. per cent*	
Dog 1. 9.0 kgm. 20 mgm. D.C.A. Removed 33.3 cc./kgm. body weight blood											
1/9	10:30 a.m.	110	84	41.8	12.6	143.5	112.2	4.4	9.3	83	
	10:52 a.m.	45	88	36.1	10.6						Completed bleeding. Weak, swaying gait
	2:00 p.m.	92	148	30.6	9.9						Refused water, appears normal
	8:30 p.m.	95	108	28.5	8.3	142.5	113.8	8.0	17.0	83	Ate full ration, drank water
1/10	10:00 a.m.	112	116	28.7	8.4						Normal
Dog 2. 13.2 kgm. 20 mgm. D.C.A. Removed 29.3 cc./kgm. body weight blood											
1/23	10:00 a.m.	106	48	41.8	11.9	142.7	109.4	5.1	18.3	79	
	11:30 a.m.	46	96	41.5	11.8						Completed bleeding, weak, listless
	2:00 p.m.	70	108	30.2	7.7						Stronger
	5:00 p.m.	57	108								Partial relapse, inactive
	8:00 p.m.	75	108	27.4	7.2	140.6	107.2	4.6	18.5	83	Alert, active, ate full ration
1/24	9:30 a.m.	88	72	24.1	6.2						Appears normal
1/25	9:30 a.m.	104	68	24.5	6.5	143.2	111.0	4.6	17.3	84	Normal
Dog 3. 10.2 kgm. 3 cc. cortical extract daily. Removed 17.3 cc./kgm. body weight blood											
3/4	9:35 a.m.	110	70	41.6	13.6	143.4	113.2	4.8	27.0	83	
	10:45 a.m.	47	72	36.0	11.8						Completed bleeding. Weak
	1:50 p.m.	75	96	30.7	10.8						Strong but listless
	10:30 p.m.	60	96	27.1	9.6	142.5	107.0	5.9	69.0	80	Refused food, alert
3/5	9:30 a.m.	46	124	30.5	10.0						In collapse. Revived with cortical extract
Dog 4. 8.0 kgm. Cortical extract withdrawn for 24 hours. Removed 15.9 cc./kgm. body weight blood											
3/11	11:00 a.m.	106	100	36.7	12.7	141.4	108.0	5.7	35.6	81	
	11:30 a.m.	44	88	32.8	12.2						Completed bleeding
	2:30 p.m.	51	156	33.4	12.5						Weak, lethargic
	4:30 p.m.	38	148	34.8	12.8	137.3	111.2	7.6	62.5	90	Died ½ hour later

within 8 to 20 hours. Animals deprived of extract for 24 hours previous to hemorrhage usually did not exhibit even this temporary rise in pressure, and the circulation failed within a shorter interval.

The D.C.A. primed dogs rapidly diluted their blood following hemorrhage, just as does the animal with intact adrenals (table 1). The hemo-

dilution observed in the animals bled while receiving maintenance doses of extract was, however, variable in extent. One dog showed a dilution equivalent to that characteristic of D.C.A treated animals (table 1, dog 3). It is of interest to note that the blood pressure of this dog was but temporarily elevated above shock levels even though the restoration of blood volume, as evidenced by hemodilution values, was of similar degree to that of the intact or D.C.A. treated animals. (Compare dog 3 with dogs 1–2, table 1.)

The animals not receiving extract for 24 hours prior to hemorrhage showed little if any signs of hemodilution at any time during the experiments.

Changes in blood chemistry characteristic of adrenal insufficiency were not found in any of the dogs subjected to hemorrhage. The blood glucose and serum chloride levels usually remained unchanged. The serum sodium concentration was usually slightly lowered, and serum potassium was elevated in some cases but not in others. A small increase in blood urea nitrogen was occasionally noted. The changes observed were small in magnitude and not always present. The data presented in table 1 indicate that blood glucose and serum electrolyte changes obviously can not be regarded as important factors in the circulatory failure resulting from hemorrhage in these adrenalectomized dogs.

II. *Ineffectiveness of D.C.A. in preventing circulatory collapse following the surgical trauma incident to a single stage bilateral adrenalectomy.* Removal of both adrenal glands at a single stage operation in the dog is almost invariably followed by death within 10 to 24 hours. Large doses of cortical extract will restore the prostrate animal to normal health and vigor; moreover, foretreatment of the animal with large amounts of extract will protect against the circulatory failure (12). However, priming the animals with D.C.A. has, in our experience, proven quite ineffectual in prolonging the survival period (13) (table 2).

The underlying basis for the rapidly fatal outcome of this type of operation is not clear. It is well established that the single stage bilateral adrenalectomy does not induce circulatory failure in such species as the rat and cat. The collapse can not be attributed to alterations in serum electrolyte pattern, hemoconcentration, and loss of body water, changes which are usually associated with adrenal insufficiency. As shown in table 3, all changes in the blood chemistry in the terminal stages of the circulatory failure following the single stage bilateral operation, are negligible, with the exception of a decline in blood sugar and this is by no means invariable. An occasional animal may also show a sharp rise in serum potassium, e.g., dog 13, table 3.

The fall in glucose may or may not attain hypoglycemic levels. It might seem that the fall in blood pressure was directly related to this decline in blood sugar. However, some animals show normal sugar values

with arterial pressures at shock levels (table 2). Moreover, there is no clear correlation between the blood sugar level and the onset of circulatory

TABLE 2

Blood pressure and blood sugar changes following single stage bilateral adrenalectomy in the dog

DOG	INITIAL		5 HOURS		9 HOURS		12 HOURS		24 HOURS		48 HOURS		
	Blood pressure	Blood sugar	Blood pressure	Blood sugar	Blood pressure	Blood sugar	Blood pressure	Blood sugar	Blood pressure	Blood sugar	Blood pressure	Blood sugar	
Untreated animals													
	mm. Hg	*mgm. per cent*	*mm. Hg*	*mgm. per cent*	*mm. Hg*	*mgm. per cent*							
5	114	87	77	82	64	82							Revived with cortical extract
6	106	82			42	64							Died at 12 hours
7	104	84			43	44							Revived with cortical extract
8	110	87	95	80	63	70							Died at 15 hours
Ave.	108	85	86	81	53	65							
Animals receiving 20 mgm. D.C.A. before operation													
9	106	89	105	83	54	68							Revived with cortical extract
10	116	84	114		42	64							Died at 13 hours
11	110	87	101		55	54							Revived with cortical extract
12	114	83	64	76	37	40							Died at 10 hours
Ave.	111	86	96	80	47	57							
Animals receiving 20 mgm. D.C.A. and intramuscular glucose													
							mm. Hg	*mgm. per cent*	*mm. Hg*	*mgm. per cent*	*mm. Hg*	*mgm. per cent*	
13	112	83	112	89	82	91	66	91	45	77	62	111	
14	110	88	108	87	73	92	66	56	50	50	73	79	
Ave.*	111	86	110	88	78	91	66	74	48	64	68	95	
Animals operated with novocaine infiltration. 1-2 mgm. D.C.A./day													
15	108	88	115	65	88	85	88		96	87	112	87	
16	110	84	112	83	99	71	99		112	80	110	85	
17	110	88	116	86	106	64	106		106	87	110	88	
18	112	77	116		106	65	106		110	75	114	75	
Ave.†	110	84	115	78	100	72	100		106	82	112	84	

* Animals sacrificed at 72 hours (see text).

† All animals survived on maintenance levels of D.C.A.

failure. To test this possible relationship between blood pressure and sugar further, two dogs were bilaterally adrenalectomized at a single stage operation in the usual way, and then hourly intramuscular injections of 10 cc.

of a 10 per cent glucose solution were given for the first twelve hours after the operation. The injections were intended to maintain the blood sugar at normal levels or above. The blood pressure decline in these two dogs was far less severe and slower than we had previously observed (table 2).

TABLE 3

Blood studies on dogs subjected to single stage bilateral adrenalectomy

DATE	TIME	BLOOD PRESSURE	PULSE	HEMATOCRIT	HEMOGLOBIN	SERUM SODIUM	SERUM CHLORIDE	SERUM POTASSIUM	BLOOD UREA NITROGEN	BLOOD SUGAR	REMARKS
		mm. Hg	*per minute*	*per cent*	*grams per cent*	*m-eq. per l.*	*m-eq. per l.*	*m-eq. per l.*	*mgm. per cent*	*mgm. per cent*	
Dog 9. 7.1 kgm. 20 mgm. D.C.A.											
12/2	11:00 a.m.	106	80	44.0	13.5	144.3	111.4	6.0	22.1	89	
12/3	12:15 p.m.	108	188							83	Completed operation
	9:15 p.m.	54	140	44.5	14.0	141.3	110.0	5.0	25.4	68	Near collapse, cortical extract injected
12/4	11:30 a.m.	96	100							84	Appears normal
Dog 11. 8.6 kgm. 20 mgm. D.C.A.											
12/6	10:00 a.m.	110	90	47.5	14.9	145.8	111.0	4.5	15.3	87	Completed operation at 11:00 a.m.
	8:00 p.m.	55	152	50.2	16.3	143.0	109.0	6.2	20.2	54	Prostrate. Died at 11:00 p.m.
Dog 13. 8.5 kgm. 20 mgm. D.C.A. Intramuscular glucose											
12/13	10:00 a.m.	112	68	42.8	14.4	143.8	111.6	6.8	15.0	83	Completed operation at 11:15 a.m.
12/14	1:00 a.m.	66	168	43.2	16.4	143.1	109.0	10.2	18.2	83	Weak, cannot walk
	10:30 a.m.	45	176	46.3	17.0	145.8	109.6	8.9	20.0	77	Weak, sacrificed at 72 hours
Dog 17. 10.2 kgm. Novocaine infiltration. 2 mgm. D.C.A./day											
2/12	10:00 a.m.	110	72	42.3	12.5	143.6	111.8	7.0	24.6	88	Completed operation at 11:55 a.m.
	9:00 p.m.	106	144	44.4	13.4	144.0	112.0	6.7	30.5	64	Active, ate full ration, normal
Dog 18. 10.0 kgm. Novocaine infiltration. 1 mgm. D.C.A./day											
2/25	11:00 a.m.	112	90	47.9	16.0	142.5	110.0	5.8	23.9	77	Completed operation at 1:00 p.m.
	11:00 p.m.	106	196	42.8	15.4	142.0	110.7	4.5	20.4	65	Strong, alert

Shock pressure levels were not reached until 20 to 24 hours after the operation. One of the two animals developed a low blood sugar at this time. Glucose injections were then continued at frequent intervals, thereby maintaining the blood sugar level normal or above for the rest of the experiment. Despite the treatment the animals remained extremely lethar-

gic, often semi-comatose, would not stand or walk, refused all food, and were sacrificed at 72 hours. The blood pressures showed some elevation from the 24 hour level, but never approached the normal. Hence, although the extra glucose definitely prolonged the survival period, the blood pressure remained low and the fatal outcome was merely postponed, the animals dying with normal, or near normal levels of blood sugar. Although it is evident that these animals did not die of hypoglycemia, disturbances in carbohydrate metabolism apparently are associated with this type of shock inducing procedure.

III. *Effectiveness of D.C.A. in preventing circulatory failure following a single stage bilateral adrenalectomy when the nerves in the vicinity of the adrenals are blocked by prococaine.* Freud *et al.* (14) and others have called attention to the presence of sympathetic plexuses in the immediate vicinity of the adrenal glands of dogs, and have emphasized the fact that, despite careful surgical technique, considerable injury and trauma to these nerves is unavoidable when the glands are removed. These authors attribute some of the symptoms generally regarded as characteristic of acute adrenal insufficiency, to injury of the nerves in the proximity of the glands.

Firor has routinely adrenalectomized dogs at a single stage operation using *spinal anesthesia* and Thorn (1) states that the animals so operated can be maintained on D.C.A. In order to test the extent to which nerve trauma was influencing the effectiveness of D.C.A. priming in our experiments, six dogs were prepared for the single stage operation in the usual manner. Before dissecting the glands previous to removal, and during the whole of the operation, the adrenals and surrounding tissues were thoroughly bathed and infiltrated with a 4 per cent novococaine solution. Approximately 10 cc. of the solution were used around each gland (tables 2 and 3). Upon recovery from the general anesthetic the dogs were alert, active and eager for food. A full ration was taken the day of operation. The blood sugar of these animals, however, still showed some decline from normal until food was taken, when it was promptly restored. No signs of circulatory failure appeared and the blood pressure fall was not over 20 mm. Hg (table 3). Whereas 20 mgm. D.C.A. used as a prophylactic foretreatment had failed to protect the dog bilaterally adrenalectomized under a general anesthetic only, the animals in which the nerve plexuses around the glands had been locally blocked could be easily maintained in excellent condition by 1 to 2 mgm. D.C.A. per day.

Discussion. The response to hemorrhage of the adrenalectomized dog primed with D.C.A. differs from that of the untreated animal in four major respects: 1. Approximately twice as much blood can be removed before the blood pressure falls to shock levels. 2. Throughout the major part of the hemorrhage, the pressure remains normal or above, while in the animal not receiving extract the pressure drops gradually but continuously from the

time of first withdrawal of blood. 3. Both during and following bleeding, the blood is rapidly diluted from extra-vascular sources in the D.C.A. primed dog whereas the untreated animal shows no blood dilution. 4. The arterial pressure spontaneously returns to normal in the primed dog, but the animal lacking hormone reserves exhibits little or no elevation of pressure from shock levels.

The circulation of the non-primed animal is apparently deficient in two important respects: 1. In the absence of hormone the dog seems unable to make adequate compensatory capacity adjustments of the arterioles. 2. He is unable to dilute the blood by withdrawal of tissue fluid into the capillaries. Both of these deficiencies are corrected by injection of extract or prevented from appearing by foretreatment with either D.C.A. or extract. The evidence suggests that the circulatory deficiencies of adrenal cortical insufficiency, as well as those which appear as a result of hemorrhage in the dog lacking adrenals, are due primarily to 1, atony of the arterioles with resulting inability to sustain a prolonged vasoconstriction; 2, atony of the capillaries with consequent pooling and stagnation of blood, anoxia and increased permeability. These changes render ineffectual the normal mechanism for fluid exchange.

It is significant that priming foretreatment of adrenalectomized dogs with D.C.A. is useless as a preventive against circulatory failure induced primarily by injury to the nervous system in the splanchnic area. We have previously called attention to the ineffectiveness of D.C.A. in preventing circulatory collapse which follows intestinal manipulation (4). Both intestinal stripping and single stage bilateral adrenalectomy are shock inducing procedures belonging in the same category since both involve extensive trauma to nervous elements in the visceral region, and both are unresponsive to D.C.A. Corticosterone, in contrast to D.C.A., will afford protection to the circulation in intestinal trauma and may also, when employed as a prophylactic, afford protection against the circulatory collapse following the single stage bilateral adrenalectomy.

Since local blocking of the nerves or spinal anesthesia effectively prevented circulatory failure from developing after the single stage operation, it is evident that afferent nerve impulses originating in the traumatized region must play an essential rôle in the etiology of the circulatory collapse resulting from this type of surgical trauma in the adrenalectomized dog.

In so far as the dog lacking adrenals is concerned, D.C.A. is an effective prophylactic only for those types of shock which are essentially non-nervous in origin.

SUMMARY

1. Desoxycorticosterone acetate, when used as a prophylactic foretreatment, protects the adrenalectomized dog against circulatory failure following hemorrhage.

2. The chief circulatory disabilities of the dog lacking adrenals and subjected to hemorrhage are: 1, inability to sustain a prolonged vasoconstriction, and 2, inability to dilute the blood. D.C.A. or extract corrects these deficiencies, or prevents their appearance when used as prophylactic foretreatment.

3. D.C.A., unlike cortical extract, is noneffective in preventing the circulatory collapse following a single stage bilateral adrenalectomy in the dog.

4. Local blocking of the nervous elements in the proximity of the adrenals before their removal prevents the onset of circulatory failure. Animals so treated can be maintained on small doses of D.C.A.

5. Circulatory failure following the single stage bilateral operation in the dog is therefore apparently due to afferent nervous impulses originating in the injured area.

6. In our experience, D.C.A. affords no protection to the circulation of the adrenalectomized dog, in those types of shock due primarily to injury and trauma to nervous elements in the splanchnic area.

REFERENCES

(1) Thorn, G. W. and H. Eisenberg. Endocrinology **25:** 39, 1939.
(2) Grollman, A. J. Pharmacol. and Exper. Therap. **67:** 257, 1939.
(3) Kendall, E. C. Proc. Staff Meetings Mayo Clinic **15:** 297, 1940.
(4) Swingle, W. W., H. W. Hays, J. W. Remington, W. D. Collings and W. M. Parkins. This Journal **132:** 249, 1941.
(5) Perla, D., D. G. Freiman, M. Sandberg and S. G. Greenberg. Proc. Soc. Exper. Biol. and Med. **43:** 397, 1940.
(6) Selye, H., C. Dosne, L. Bassett and J. Whittaker. Canad. M. A. J. **43:** 1, 1940.
(7) Weil, P., G. B. Rose and J. S. L. Browne. Canad. M. A. J. **43:** 8, 1940.
(8) Selye, H. and C. Dosne. Lancet **239:** 70, 1940.
(9) Parkins, W. M. This Journal **107:** 518, 1934.
(10) Swingle, W. W., W. M. Parkins, A. R. Taylor and H. W. Hays. This Journal **119:** 684, 1937.
(11) Swingle, W. W., J. J. Pfiffner, H. M. Vars and W. M. Parkins. This Journal **107:** 259, 1934.
(12) Swingle, W. W. and W. M. Parkins. This Journal **111:** 426, 1935.
(13) Parkins, W. M., W. W. Swingle, J. W. Remington and V. A. Drill. This Journal **133:** P408, 1941.
(14) Freud, J., I. Ulydert and M. L. Waterman. Endocrinology **22:** 497, 1938.

TERMINAL CEREBROSPINAL FLUID PRESSURE VALUES IN VITAMIN A DEFICIENCY[1]

L. A. MOORE AND J. F. SYKES

From the Sections of Dairy Husbandry and Physiology, Michigan State College

Accepted for publication July 18, 1941

In a previous paper Moore and Sykes (1940) reported that a deficiency of vitamin A in the ration of calves produced an increased cerebrospinal fluid pressure accompanied by papilledema, nyctalopia, syncope and incoördination. The addition of crystalline carotene to the ration caused these disturbances to disappear and the cerebrospinal fluid pressure gradually returned toward normal. In the previous work the cerebrospinal fluid pressure was not permitted to increase markedly since it was desired to restore the pressure to normal levels as quickly as possibly by carotene therapy. It is the purpose of the present paper to report values for the pressure of the cerebrospinal fluid where the animals were permitted to proceed to the terminal stage of vitamin A deficiency.

EXPERIMENTAL. The animals used in this experiment had been maintained on various levels of carotene to determine the amount of carotene required to prevent the development of an increased cerebrospinal fluid pressure in the growing calf. In the experiments reported here the source of carotene was removed from the ration of these animals in order to allow the various individuals to develop severe vitamin A deficiency symptoms.

The low carotene ration consisted of skimmed milk and a concentrate mixture consisting of 240 pounds barley, 180 pounds rolled oats, 180 pounds wheat bran, 60 pounds linseed oil meal and 8 pounds salt. This ration contained sufficient carotene to supply two to four micrograms per kilogram of body weight per day. Wood shavings were used as bedding.

Blood plasma carotene determinations were made each week according to the method of Moore (1939). Cerebrospinal fluid pressures were obtained by puncture into the subarachnoid space. The insertion was made through the dorsal opening in the atlanto-occipital articulation. No anesthetic was used and the records were obtained with the animals standing quietly.

Animal I was an Ayrshire female which since four months of age had been receiving carotene from alfalfa leaf meal at a rate of 60 micrograms of

[1] Journal Article no. 534 (n.s.).

carotene per kilo of body weight. At this intake of carotene the cerebrospinal fluid pressure increased to about double the normal value and some slight swelling of the nerve head was observed. Otherwise the animal appeared normal. At 498 days of age the alfalfa meal was removed from the ration and at 511 days a convulsive seizure was noted. Convulsions were seen periodically during the succeeding period and could sometimes be brought on by excitement caused by such procedures as drawing blood samples. During the last two weeks this animal moved around very little and had some difficulty getting up. Diarrhea developed and a marked

TABLE 1

Terminal cerebrospinal fluid pressure values in vitamin A deficiency

AGE	PLASMA CAROTENE	CEREBROSPINAL FLUID PRESSURE	AGE	PLASMA CAROTENE	CEREBROSPINAL FLUID PRESSURE
Animal I			Animal III		
days	*micrograms per ml.*	*mm. of saline*	*days*	*micrograms per ml.*	*mm. of saline*
431	1.0	190	330	0.35	125
458	1.0	190	378	0.35	120
501	0.7	205	398	0.35	140
536	0.2	345	428	0.25	170
563	0.12	380	566	0.10	360
594	0.01	520	583	0.10	580
605	0.01	600	604	0.03	400
612	0.02	460	640	0.02	310
Animal II			Animal IV		
726	0.7	110	431	0.81	220
755	0.2	125	466	0.95	300
825	0.05	280	500	0.87	210
831	0.05	470	535	0.19	290
			563	0.07	420

papilledema was present. At 620 days of age the animal was unable to rise and was sacrificed in order to save the tissue for pathological study. The principal results are shown in table 1 from which it will be noted that as the plasma carotene dropped, the cerebrospinal fluid pressure increased and reached a maximum of 600 mm. of saline. A week later the pressure dropped to 460 mm.

Animal II was a Holstein male which had been receiving 120 micrograms of crystalline carotene dissolved in cottonseed oil per kilo of body weight. The carotene was removed from the ration at 726 days of age after which incoördination and convulsions were apparent at 771 days. Papilledema was evident at 801 days, the animal was unable to rise at 831 days of age

and was sacrificed at that time. The principal results are shown in table 1. It will be noted here that the cerebrospinal fluid pressure reached a maximum of 470 mm. after carotene was removed from the ration. Animal III was also a Holstein male which had been receiving 60 micrograms of carotene per kilo of body weight. The alfalfa was removed from the ration at 394 days of age. Papilledema and incoördination developed at 566 days and the animal died at 648 days. The principal results are shown in table 1. It will be noted that this animal like animal I showed a terminal drop in cerebrospinal fluid pressure after developing a maximum pressure of 580 mm.

Animal IV was an Ayrshire male which had been receiving 45 micrograms of carotene per kilo of body weight and had developed an elevated pressure at this level of carotene intake. The alfalfa was taken out of the ration at 498 days of age after which the animal showed marked papilledema and incoördination at 563 days. It was necessary to sacrifice the animal at 571 days of age. When carotene was withdrawn from the ration the pressure increased to 420 mm.

It was possible to increase the high pressure of these animals still further by excitement such as slapping them on the back. This excitement often caused approximately a two-fold increase. In one instance, an increase to 1060 mm. from a previous level of 460 mm. was observed and in another a pressure of 560 mm. developed during excitement from a previous level of 290 mm.

Discussion. The results show that when young bovine are placed on a low carotene ration the cerebrospinal fluid pressure may attain values from 400 to 600 mm. of saline. This would be about 4 to 6 times the normal value. These values were obtained with animals at the terminal stage of vitamin A deficiency. In two cases there was a terminal drop in pressure from a previously higher level. Usually the animals in this condition had very little appetite, showed diarrhea and were more or less in a moribund state so that the drop was not surprising.

The increase in cerebrospinal fluid pressure was always accompanied by a marked papilledema, incoördination and periods of syncope. The condition of syncope often proceeded to a state of convulsive seizure during which respiration ceased for short periods of time. It was felt that the syncope and convulsive like seizures were due to a cerebral anemia because of the increased cerebrospinal fluid pressure. The fact that excitement often caused these seizures further indicates that this may be true since it has been shown that a marked increase in pressure accompanies periods of disturbance.

The particular cause of the increased cerebrospinal fluid pressure in vitamin A deficiency in the bovine has not been found. Pathological study of the choroidal plexus and arachnoid villi, colloidal osmotic pressure measure-

ments of blood plasma and various blood and urine analysis have not shown any abnormality which could be related to the raised pressure. In this connection, however, Wolbach and Bessey (1940) have reported a relative overgrowth of the central nervous system in vitamin A deficiency in rats. They report extensive and striking herniations of the nerve roots into the intervertebral foramina, herniations into the bodies of the vertebrae, an increase in size of the contents of the cranium as evidenced by *a*, the presence of herniations of the cerebrum, cerebellum and posterior colliculus; *b*, distortion of the brain; *c*, changes in the contours of the fossae of the floor of the skull. If there is a relative overgrowth of the central nervous system in the bovine such as reported by Wolbach and Bessey in rats it could easily account for the increased cerebrospinal fluid pressure reported here. Evidence of bony changes in the cranial cavity in vitamin A deficiency in calves has already been reported by Moore, Huffman and Duncan (1935) and Moore (1939) in which a blindness was produced by a stenosis of the bony optic canal through which the optic nerve passes. In the previous paper it was reasoned that the bony malformations in calves were possibly due to the increased cerebrospinal fluid pressure.

Wolbach and Bessey (1940) have expressed belief that the relative overgrowth of the central nervous system is a growth phenomenon. However, Moore (1941) has shown that papilledema due to vitamin A deficiency will develop in mature cows which might indicate that the increase in the volume of the central nervous system was not entirely confined to the growth period. De Schweinitz and De Long (1934) have presented evidence that a perivascular edema of the brain tissue accompanies papilledema and blindness in calves which may indicate that a similar increase in volume of the cranial contents is the cause of the raised pressures observed in vitamin A deficiency. It seems likely that the increased pressure is due to an increased volume of the cranial contents caused either by a relative overgrowth as indicated by Bessey and Wolbach or to an actual increase in the volume of the cranial contents.

SUMMARY

1. Terminal cerebrospinal fluid pressure values of 400 to 600 mm. of saline were recorded in young bovine fed a vitamin A deficient ration.

2. The possible cause of the increased pressure is discussed.

REFERENCES

DeSchweinitz, G. E. and P. De Long. Arch. Opthal. **2:** 194, 1934.
Moore, L. A., C. F. Huffman and C. W. Duncan. J. Nutrition **10:** 409, 1935.
Moore, L. A. and J. F. Sykes. This Journal **130:** 684, 1940.
Moore, L. A. J. Dai. Sci. **22:** 501, 1939.
J. Nutrition **17:** 443, 1939.
In process of publication.
Wolbach, S. B. and O. A. Bessey. Sci. **91:** 599, 1940.